Wild Plants, Mushrooms and Nuts

Functional Food Properties and Applications

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Preface

The use of healthy ingredients is a natural way of preventing diseases and contributes to the increased use of natural matrices. This book focuses on the nutritional, chemical, and biological properties of natural matrices from the Iberian peninsula, mainly food products such as wild plants, mushrooms, chestnuts, and almond.

Society's attitude to food, as a natural and inevitable necessity, has altered in line with changes in social conditions and development of technology. Current consumers are interested in the composition, properties, safety, and health effects of food products. The desire to consume foods with high biological value from natural origins poses a huge challenge for modern food science and industry. In addition, the recent consumer interest in chemopreventive nutrition has increased the choice of food products (functional foods) with specific components (bioactive compounds). The current increase in the adoption of more active and healthy lifestyles needs to be followed by a concomitant response from all players in the food chain. The knowledge contained in this book will allow scientists and, in the longer term, lay members of society to gain a better understanding of the value that these products exhibit, focusing on their nutritional and chemical composition, bioactivity, and potential as functional foods.

Ongoing research on selected products will lead to a new generation of foods, and will promote their nutritional and medicinal use. Public health authorities consider prevention and treatment with nutraceuticals a powerful instrument in maintaining and promoting health, longevity, and life quality. The beneficial effects of nutraceuticals will undoubtedly have an impact on nutritional therapy; they also represent a growing segment of today's food industry. Therefore wild plants, mushrooms, and nuts have become interesting food products due to the increasing interest in the concept of "functional foods" with "health benefits."

Wild Plants, Mushrooms and Nuts: Functional Food Properties and Applications is a compendium of current and novel research on the chemistry, biochemistry, nutritional and pharmaceutical value of traditional food products, which are becoming more relevant in our current diet, for developing novel health foods and in modern natural food therapies. Topics covered range from their nutritional value, chemical and biochemical characterization, to their multifunctional applications as food with beneficial effects on health, through their biological and pharmacological properties (antioxidant, antibacterial, antifungal, and antitumor capacity, among others).

1
Introduction: The Increasing Demand for Functional Foods

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1.1 Food Patterns: A Cross-sectional Approach and Brief Overview

Primitive societies often lack resources but have always emphasized the role of nutrition in maintaining good health and wellbeing (Balch 2006; Murray & Pizzorno 2005, 2012). So, the idea of a balanced and wholefood-enriched diet to ensure homeostasis and improve life expectancy is not new.

Concomitantly with the intensification of the globalization process and advances in the food industry, a pronounced increase in public health problems has been observed. Health-related economic and social costs have risen to represent a significant percentage of worldwide expenditure (American Dietetic Association 2009; Arvanitoyannis & Houwelingen-Koukaliaroglou 2005). Public health problems affect all sectors of society – elderly, adults, children, and adolescents. Therefore, the deployment of prevention strategies seems to be essential, not only to avoid the progression of this worldwide problem but also to try and restore the balanced food patterns and proper lifestyle of individuals.

Infectious diseases were the most frequent causes of morbidity and mortality among the first civilizations, mainly attributed to poor hygiene conditions, and efforts were made to reduce the incidence of outbreaks of infection and epidemics. Nowadays, research is

carried out to find even more effective and specific chemical drugs, allegedly able to treat modern disorders, although most of them can be eradicated just through lifestyle modifications. Metabolic disorders and related problems are some of the most important current contributors to human morbidity and mortality. Overweight and obesity, considered the epidemic of the 21st century, increasingly affects all age groups, with children being the most vulnerable (Arvanitoyannis & Houwelingen-Koukaliaroglou 2005; Bagchi 2006).

Hippocrates said that "whatever be the father of a disease, the mother is always a bad diet" (Longe 2005; Murray & Pizzorno 2005, 2012). Linked with the increasing incidence of metabolic disorders has been a demand for new food products. Addictive behavior, feelings of pleasure, and palatability are the main determinants of food choices in modern civilization (Balch 2006; Jauho & Niva 2013; Murray & Pizzorno 2005). Thus, it is not surprising that rates of chronic disorders, most of them food pattern related, have reached epidemic levels, and are likely to increase in the coming years.

1.2 Nutrition and Health: Facts and Tendencies

1.2.1 Evidence-based Medicine: Past to Present

There are numerous reports and historical manuscripts proving data about the applications of botanicals and plant food preparations, for both nutritional and medicinal uses (Khan & Abourashed 2010; Longe 2005; Murray & Pizzorno 2012; Vanaclocha & Cañigueral 2003). Traditional medicine dates back to the dawn of human civilization; primitive societies used botanical preparations and even plant food derivatives for medicinal, culinary, preservative, and aromatizing purposes (Ferreira *et al*. 2009; Junio *et al*. 2011; Rubió *et al*. 2013; Sahib *et al*. 2013; Spelman *et al*. 2006; Sung *et al*. 2011; Viuda-Martos *et al*. 2010; Zheng & Wang 2001). Numerous attributes were

conferred on ethnopharmacological preparations, which have been increasingly validated through epidemiological, preclinical, and even clinical studies (American Dietetic Association 2009; Ferguson 2009; Sung *et al*. 2011; Viuda-Martos *et al*. 2010). Primitive societies gained knowledge about identification, culture and ideal harvesting conditions, indications, contraindications, side-effects, and toxicity of natural products, as well as recommended dosages (Balch & Stengler 2004; Balch *et al*. 2008; Murray & Pizzorno 2012; Vanaclocha & Cañigueral 2003). Therefore, early civilizations discovered a multitude of natural product potentialities and applications but because of the lack of scientific evidence, they could not pinpoint the main responsible active principles. More recent researchers, aiming to deepen knowledge in this area, have often used previous findings to guide their current studies.

In relation to the nutritional and medicinal use of natural products, it is important to highlight direct consumption as part of the daily diet but they are also used as flavorings, preservatives, flavor intensifiers, and so on (Balch 2006; Balch & Stengler 2004; Khan & Abourashed 2010; Longe 2005; Murray 2004; Murray & Pizzorno 2005; Vanaclocha & Cañigueral 2003). Research has been focused not only on their health improvement effects but also their organoleptic properties.

In spite of cultural, ethnic, and religious patterns, the importance of a balanced diet is clearly evident. Since earliest times, human beings have understood that a balanced diet is crucial to survival and to maintain good health and wellbeing (Balch 2006; Murray & Pizzorno 2005, 2012). Dietary information has been passed through generations. The difference between edible and nonedible products was determined over time, including toxic potential and unpleasant side-effects. Different forms of preparation and cooking were developed, including the use of botanicals as herbs and spices to improve taste and general acceptability of food. At the same time, ways to improve the shelf-life of numerous products were found, and to prevent the occurrence of organoleptic changes (Balch & Stengler 2004; Khan & Abourashed 2010; Murray 2004; Murray & Pizzorno 2005). The discovery of the prophylactic and therapeutic potentialities of botanicals required thousands of years of observation and analysis. There are no doubts about the direct

impact of a balanced diet and lifestyle to ensure good health and wellbeing. In fact, 2500 years ago, Hippocrates highlighted the real value of nutrition, of health-conscious eating habits, and adequate preparation of meals as important contributors to long-lasting wellbeing (American Dietetic Association 2009; Biziulevičius & Kazlauskaitė 2007; Sung *et al*. 2011; Wegener 2014).

Over the years, the number of studies into botanical functionality, natural products, and their bioactive potential has increased in an exponential manner (Balch 2006; Balch & Stengler 2004; Balch *et al*. 2008). Different civilizations possess characteristic health doctrines and therefore different ways to prepare meals, mainly derived from perceptions about the intellectual, physical, energetic, therapeutic, and culinary applications of food (Kaput 2008; Murray 2004; Murray & Pizzorno 2005, 2012). With the globalization process, many local food habits have been changed and intercultural relationships established (Murray & Pizzorno 2005, 2012). Not all of this was bad but in relation to health and nutrition, a positive correlation between modified food patterns and prevalence of diseases and organic disorders has been increasingly stated over recent years (Arvanitoyannis & Houwelingen-Koukaliaroglou 2005; Fenech *et al*. 2011; Jones & Varady 2008). Neurodegenerative, cardiovascular, metabolic and immune diseases, and aging-related conditions, represent the most frequent and serious disorders, at a public health level (Ergin *et al*. 2013; Murray & Pizzorno 2012; Nasri *et al*. 2014).

It is important to bear in mind that geographical, cultural, and ethnic differences produce pronounced variations at genetic, molecular, and organic levels (Balch *et al*. 2008; Longe 2005; Murray & Pizzorno 2005, 2012). People living in distinct areas have specific genetic patterns and therefore different metabolic pathways and related responses to ingested foods (Fenech *et al*. 2011; Ferguson 2009; Kaput 2008). There are increasing evidences related to the effects of the interaction between foods and the individual's genome (nutrigenomics), leading to consequences at the level of the phenotype. This explains why a particular dietary practice may be appropriate for one individual and inappropriate for another (Fenech *et al*. 2011; Kaput 2008).

On the other hand, the effects of genetic variations on dietary responses (nutrigenetics) have also been increasingly reported (Fenech *et al*. 2011). Based on these factors, increasingly detailed studies have been developed to improve the correct usage of plant food products, to discover their main active principles and mechanisms of action, and to widen perspectives about their use not only for prophylactic but also therapeutic purposes. Although genetics have some influence, environmental and lifestyle patterns are the main triggering factors which disturb organic homeostasis and thus affect the occurrence of disorders and diseases.

1.2.2 Modern Food Patterns: An (Un)Healthy Yield

Bearing in mind the previous explanations, and considering the increasing worldwide health-related economic and social costs, relating to medical devices, drug discovery, and other pharmacological advances (American Dietetic Association 2009; Arvanitoyannis & Houwelingen-Koukaliaroglou 2005; Bagchi 2006; Bigliardi & Galati 2013), research and industrial modifications have been increasingly implemented in attempts to control this serious problem. With the increasing rates of chronic disorders, more specific and more effective drugs needed to be synthesized, tested, and evaluated, to assess their possible application in humans (Holst & Williamson 2008; Khan *et al*. 2013; Li *et al*. 2014; Nasri *et al*. 2014). Experimental drug studies need to be conducted for proper evaluation of their side-effects and related toxicity. However, much more important than medical and/or chemical drug interventions is the effect of dietary patterns and lifestyle (Balch 2006; García-Elorriaga & Rey-Pineda 2013; Kaput 2008; Sung *et al*. 2011).

Currently, several foods have been shown to be potent contributors to improving the health status and wellbeing of consumers and, at the same time, are able to reduce the incidence of social, and economic costs of noncommunicable and disabling disorders (Das *et al*. 2010).

The use of foods with known beneficial effects is important to improve the shelf-life and safety of numerous foodstuffs, and consequent reduction of the likelihood of side-effects, and also

their organoleptic properties (Bagchi 2006; Bigliardi & Galati 2013; Jones & Varady 2008). Furthermore, in some instances, those products/substances can modify the acceptability of other products, making them more attractive. Herbs and spices (Barros *et al*. 2011; Morales *et al*. 2013; Rubió *et al*. 2013; Viuda-Martos *et al*. 2010), mushrooms (Ferreira *et al*. 2009; Heleno *et al*. 2015; Ribeiro *et al*. 2015), and oilseed fruits (Contini *et al*. 2012; Preedy *et al*. 2011; Siqueira *et al*. 2012) have been extensively studied and used not only to improve the nutritional value and shelf-life of many other products but also for their organoleptic properties, among many other benefits, some of which are still being investigated. It is interesting to highlight that, being themselves already considered functional foods, they also contribute to the health benefits, applications, and claims of many other food products (Arvanitoyannis & Houwelingen-Koukaliaroglou 2005; Bigliardi & Galati 2013; Siró *et al*. 2008).

Thus, functional foods are important in the daily consumption of a balanced diet, and also for their inclusion in many other edible products. The verification of the bioactive potential and other qualities of modified food products, and general consumer acceptability, are among the most promising fields in biotechnological and food industrial research.

1.3 Functional Foods Diversity and Related Applications: A World of (Un)Explored Biofunctionalities

Over the years, the study of the bioactive properties of edible matrices has increased exponentially, in association with scientific evidence that confirms their wide variety of applications and benefits that were promoted by folk medicine and primitive societies but lacked solid foundation and scientific validation (Balch 2006; Murray 2004; Murray & Pizzorno 2005).

Nutritional composition, in terms of proteins, lipids, carbohydrates, dietary fibers, vitamins, minerals, and other micronutrients, and also secondary metabolites, mostly existing in

vestigial amounts, has received special attention (Mishra & Tiwari 2011; Murray & Pizzorno 2005; Rubió *et al*. 2013). Observational, longitudinal, and cohort studies have been conducted, in which not only nutritional but also therapeutic properties were observed (Balch 2006; Murray & Pizzorno 2005). The positive effects of the Mediterranean diet on cardiovascular health have been determined, through preferential consumption of wholegrains, seeds and nuts, fruits and vegetables, and cold-pressed oils (Murray & Pizzorno 2005; Yildiz 2010). These foods are extremely rich in beneficial nutrients, such as soluble and insoluble dietary fibers (promote healthy bowel function, improve glycemic and blood cholesterol index, etc.), mono- and polyunsaturated fatty acids (act as neurocognitive, cardiovascular, endocrine health improvers, etc.), vitamins and minerals (essential nutrients which promote enzymatic and metabolic function, etc.) (Balch 2006; Murray & Pizzorno 2005). However, there are many other chemical constituents that can improve these functions and provide other bioactive properties.

Antioxidant, antimicrobial, antitumor, antiseptic, antiinfectious, antiinflammatory, hepatoprotective, antidiabetic, and neuroprotective effects are among the most commonly assessed bioactive properties of the minor constituents of natural matrices. Intense investigation still continues in this field; numerous bioactive constituents have already been identified, including their mechanisms of action and biochemical interactions, but there are thousands of secondary metabolites that still remain unknown, and therefore need to be explored (Arif *et al*. 2009; Choudhary & Atta-ur-Rahmant 1999; Coman *et al*. 2012; Mishra & Tiwari 2011; Murray & Pizzorno 2005). The increasing demand to assess the beneficial effects of foods and their bioactive molecules is largely driven by increasing evidence of side-effects and adverse reactions produced by pharmaceutical drugs (Balch *et al*. 2008; Coman *et al*. 2012; García-Elorriaga & Rey-Pineda 2013; Palombo 2011; Sangamwar *et al*. 2008). In fact, many synthetic molecules were previously isolated from natural sources and then synthesized for large-scale production.

In the last decade, different terms have been adopted for natural products with specific and recognized functions in the human body. Although no general consensus has yet been established, the

terms “functional food” and “nutraceuticals” have become a focus of attention for the scientific community and consumers (Bagchi 2006; Murray & Pizzorno 2005; Nasri *et al.* 2014). A functional food is commonly thought of as a food included in the normal diet which has one or more target functions in the human body, being able to improve the health status and/or reduce the likelihood of disorders occurring (Bagchi 2006). Such food should provide those benefits in the amount that can be expected to be ingested in the daily diet; therefore, they cannot be pills, capsules, syrups, etc. but should be part of a healthy food pattern (Bagchi 2006). A functional food can also be a natural/whole/unmodified food or food component in which a specific constituent has been added and/or removed by biotechnological or technological processes (Bagchi 2006; Nasri *et al.* 2014). Furthermore, it can also undergo various manipulations in order to modify or alter the bioavailability of specific constituents, focused on the improvement of its health benefits (Bagchi 2006; Bigliardi & Galati 2013; Das *et al.* 2010).

Overall, despite all these advances, the field of functional foods research still remains a real challenge. However, to improve the accuracy and applicability of current findings, health professionals, nutritionists, food industries, and regulatory toxicologists should work together, aiming for the goals of health promotion and disease prevention.

1.3.1 Food and Dietary Supplements, Botanicals, and Nutraceuticals: Clarifying Misinterpreted Concepts

The beneficial effects of diet-specific components and related scientific studies that support these findings lead to increasing interest in developing more specific tools and related technologies to improve and maintain an optimum level of health and wellbeing. However, several misinterpretations still exist. One is related to the correct definition of food supplements, botanicals and related preparations, and nutraceuticals.

The term “nutraceutical” is a combination of the terms “nutrition” and “pharmaceutical,” and refers to food/botanical ingredients or

extracts that have defined physiological effects (Bagchi 2006; Nasri *et al*. 2014). So, in general, nutraceuticals are substances which provide beneficial effects not when consumed as part of a normal diet (functional food), but when consumed in unitary pharmaceutical doses, such as tablets, capsules, syrups, and so on (Bagchi 2006; Espín *et al*. 2007).

On the other hand, the term "food supplement" refers to concentrated sources of nutrients and other specific substances that have nutritional and/or physiological effects, in which the main goal is to supplement/enrich the normal diet. Food supplements may be beneficial to correct nutritional deficiencies, to maintain an adequate intake of certain nutrients or even to ensure a healthy status. But it is also important to be aware that in some cases, excessive intake of vitamins, minerals, and other vestigial micronutrients may be harmful, inducing undesired side-effects and even toxicity. Following the current nutritional guidelines is of the utmost importance in order to ensure their correct and safe use in food supplements (EFSA 2015a).

Lastly, many health claims have been put forward for botanicals and plant-derived preparations, typically labeled as natural foods, most of which arise from their ancient use by primitive societies. In line with the scientific evidence on their health benefits, they have become increasingly available in the EU, in the form of food supplements, being easily found in pharmacies, supermarkets, and specialized shops, as well as in the internet (EFSA 2015b).

1.4 Functional Foods Versus Bioactive Molecules: Hierarchies and Regulatory Practices

Over the years, numerous concepts and definitions have been progressively established in order to distinguish the latest advances in the field of health-related nutrition. In the first instance, an increasing number of foodstuffs present on their labels several "claims," e.g. messages or representations, which are not mandatory under EU or national legislation, including pictorial, graphic or symbolic representations which state, suggest or imply

that a food has particular characteristics (European Regulation (EC) No 1924/2006). Apart from the vitamins and minerals, including trace elements, amino acids, essential fatty acids and dietary fibers, there are other substances present in natural matrices (e.g. plants and herbal extracts) that are also able to confer nutritional or physiological benefits. However, as foods with these types of claims tend to be perceived by consumers as having superior health advantages compared with other food products, general principles and strict rules should be applied to all food claims in order to ensure a high level of protection, information, and equal conditions of competition for the food industries, as well as encouraging consumers to be aware of making choices which directly influence their total intake of individual nutrients or other substances in a way which might run counter to scientific advice. In line with this, the concept of a "health claim" was established, which refers to any claim that states, suggests or implies the existence of a relationship between a food category, a food or one of its constituents, and good health (European Regulation (EC) No 1924/2006). Further, the concept of a "health food" also deserves particular mention, defining a food product that possesses "special nutritious elements" or "special healthcare abilities," being able to improve health and wellbeing and/or to reduce the occurrence of disorders/diseases (Bagchi 2006).

However, the labeling of a particular food product as a health food carries several conditions, including that it should have clearly identified bioactive constituents that exert beneficial effects, upheld by proper scientific support and proofs. In addition, it must be safe and its consumption should be harmless to humans, and duly supported by toxicological studies (Bagchi 2006). Finally, if it is not possible to identify the specific bioactive components, all the beneficial effects should be clearly listed and properly supported by literature (Bagchi 2006). Then, the relevant health authority will evaluate all the methodologies used to assess the real efficacy and safety of the foods and their specific bioactive constituents in order to approve and permit their qualification/labeling as a health food (Bagchi 2006; Lupton 2009).

However, approval of a food product as a health food does not mean its qualification as "functional food." As previously highlighted, the definition of a functional food, to a certain extent,

overlaps with the health food definition but after the acceptance of a particular food product as a health food, other regulatory procedures are necessary to authorize its labeling as a “functional food” (Bagchi 2006; Lupton 2009). In both cases, and despite health claims attributed to specific foods through proper scientific assessments and proofs, not all regulatory authorities permit the free labeling of health allegations. In the EU, health claims are only permitted if the labeling includes a statement indicating the importance of a varied and balanced diet and a healthy lifestyle; the quantity of the food and pattern of consumption required to obtain the claimed beneficial effect; a statement addressed to individuals who should avoid using the food; and an appropriate warning for products that are likely to present a health risk if consumed in excess (European Regulation (EC) No 1924/2006). For example, in contrast with the United States and some European regulations, the Health Food Control Act (HFCA) in Taiwan does not allow a direct link to be made between a food bioactive ingredient and a particular disease; among other explanations, some nongovernmental Taiwanese institutions state that food health products should be evaluated as a whole, and that the use of excessive amounts of adverse ingredients in their formulation should be restricted (Arvanitoyannis & Houwelingen-Koukaliaroglou 2005; Bagchi 2006; Lupton 2009). This rule makes sense because often, it is not only a specific bioactive constituent that is responsible for the supposed health benefits but all of the consumed food constituents. Whole matrices play a more important role in maintaining the health status of consumers than a single ingredient. Currently, this rule is implemented in the US as a prerequisite for foods which carry a health claim on the label (Bagchi 2006; Jauho & Niva 2013; Lupton 2009).

In general, health foods, including functional foods, claim that their use maintains or even improves a specific health status. There are numerous chemical constituents present in the whole matrices, some of which provide a greater or lesser contribution to their biological activity (Arvanitoyannis & Houwelingen-Koukaliaroglou 2005; Bagchi 2006; Doyon & Labrecque 2008; Jauho & Niva 2013). Therefore, before promoting a special food or derived ingredient as better and healthier, it is of the utmost importance to identify all the bioactive constituents, including their mechanism of action, biochemical interactions, and other

specific parameters, which allows their full recognition, guides future researches, and at the same time provides scientific evidence for their regulatory approval and ensures the correct and safe dosage. These scientific proofs are crucial to the regulatory evaluation, and are derived from *in vitro* but mainly *in vivo* studies and clinical trials.

In respect to food consumption, claims should not be interpreted in a unidirectional manner. On one hand, there are no foods with approved health claims without proper scientific support, but on the other hand, hasty conclusions should be avoided. Bioactive molecules exist to a large extent in many food products but it is important to select foods rich in these constituents. In this way, not only the specific health benefits conferred by these selected components but also other additional effects (e.g. provided by the biochemical interactions and synergisms between the pool of chemical constituents) will be achieved (Bagchi 2006; Mukherjee & Houghton 2009; Yildiz 2010). Several experiments have shown that the most pronounced benefits are obtained by using the whole matrices rather than isolated/individual constituents.

1.5 Challenges and Opportunities: A Multidimensional Perspective

In line with current research, a multitude of health benefits provided by the consumption of plants, mushrooms, nuts, and other whole matrices have been increasingly reported and are recommended by public health guidelines (American Dietetic Association 2009; Balch 2006; Fenech *et al.* 2011; Ferguson 2009). However, despite current achievements, several problems still exist.

There are no doubts about the real potential of naturally occurring edible products, but strategies to improve their biological availability, applicability, consumption strategies, etc. are not completely established. Additionally, for the majority, the active principles, modes of action, and therapeutic properties have not been adequately determined. So, intense work is still being carried out. Different strategies need to be implemented in order to

improve the applicability and potential of natural matrices and their bioactive components, including their potential for improving the nutritional and possibly therapeutic values of other food matrices (Barroso *et al.* 2014; Bigliardi & Galati 2013; Nasri *et al.* 2014; Sadaka *et al.* 2013). Microencapsulation techniques help to ensure the sustained release of active principles derived from plants, foods, and even whole matrices, in order to improve their metabolic and physiological functions and at the same time reduce the occurrence of side-effects (Barroso *et al.* 2014; Bigliardi & Galati 2013; Ribeiro *et al.* 2015; Sadaka *et al.* 2013).

Another interesting biotechnological advance in the food industry is the inclusion of plants (namely herbs and spices) in different food matrices, e.g. dairy products, such as milk derivatives (Caleja *et al.* 2015a, 2015b; Carocho *et al.* 2015a), biscuits, etc. (Carocho *et al.* 2014, 2015b) to improve their shelf-life and biological potential, making them functional foods. This also helps to reduce the use of synthetic preservatives, some of which have medium- and long-term side-effects, acting as triggers for the occurrence of numerous disorders, and which even compete with numerous active principles, reducing their bioavailability and related bioefficacy. Moreover, it is also possible to improve their digestibility and organoleptic characteristics (some are marketed as gourmet products).

These types of research are time-consuming and complex procedures, in which the results obtained are not always what was expected.

Other factors should be considered, including:

- the use of whole matrices and most effective parts (taking into consideration their origin: commercial vs wild sources)
- the use of isolated/individual chemical constituents and mixtures
- different dosages/concentrations
- initial vs final organoleptic properties
- bioavailability and incremental changes.

Therefore, detailed experiments need to be developed to assess and

confirm the real *in vivo*, and to a lesser extent *in vitro*, bioactive potential of upcoming advances in the field of functional foods and nutraceuticals. Furthermore, many other natural matrices should be explored and their viability, stability, and feasibility duly analyzed *in vitro*, including determination of the edible parts and assessment of their mode(s) of action and related pharmacokinetic and pharmacodynamic parameters, in order to infer their subsequent *in vivo* application.

In short, despite all the currently available reports, the biotechnological and food technological areas still require intense research and innovation. The main goals of global research institutions are to provide more and better products to the human population, aiming to improve their health and wellbeing and, at the same time, to prevent the occurrence of diseases and disorders. However, it should never be forgotten that balanced nutrition is the key to an optimum health status.

1.6 Conclusion

With the current advances in the fields of basic and applied nutrition, numerous aspects have been progressively implemented to ensure an adequate level of organization, regulation, and certification of edible foods with claimed beneficial effects. Functional foods, for example, have gained particular attention not only from consumers but also biotechnological, chemical, pharmaceutical, and food industries, and also from medical and scientific communities. Nonetheless, with this increasing demand, it is crucially important to ensure the safety of the products and protection of consumers. Health claims and other nutritional and physiological attributes of plant food-derived formulations are increasingly found on food labels, although several requirements are mandatory. Thus, new interesting challenges and opportunities have opened up. Firstly investigated for their nutritional value, chemical composition, and health benefits, food products are currently being used to carry out multiple studies, varying from the molecular and genetic levels to biotechnological and industrial applications.

Due to the deepening of knowledge in this area and new perspectives arising, this is an almost infinite area of research, given the vast quantity of natural substances. Many studies can be undertaken to assess their biological potential; to discover their chemical composition and active principles responsible for observable bioactivities; to assess mechanisms of action, molecular and biochemical interactions, possible toxicity, and so on. Industrial and technological applications are also experiencing a rapid progress. For example, initially, naturally occurring foodstuffs with prestigious health benefits (functional foods) were marketed for direct consumption and increasingly privileged by consumers; then, a modified presentation was developed and industrial processes applied to improve their biological potential and bioavailability. Currently, they are exhaustively tested and their ability to improve the nutritional value and bioactive potential of many other daily foods have been determined. Short- and medium-term studies and the obtained results from the organoleptic evaluations by consumers indicate a promising future in this area.

Although much more remains to be done, one factor is certain: nature can provide all the necessary tools to ensure the wellbeing and longevity of the human population.

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2
The Numbers Behind Mushroom Biodiversity

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2.1 Origin and Diversity of Fungi

Fungi are difficult to preserve and fossilize and due to the poor preservation of most fungal structures, it has been difficult to interpret the fossil record of fungi. Hyphae, the vegetative bodies of fungi, bear few distinctive morphological characteristicss, and organisms as diverse as cyanobacteria, eukaryotic algal groups, and oomycetes can easily be mistaken for them (Taylor & Taylor 1993). Fossils provide minimum ages for divergences and genetic lineages can be much older than even the oldest fossil representative found. According to Berbee and Taylor (2010), molecular clocks (conversion of molecular changes into geological time) calibrated by fossils are the only available tools to estimate timing of evolutionary events in fossil-poor groups, such as fungi.

The arbuscular mycorrhizal symbiotic fungi from the division Glomeromycota, generally accepted as the phylogenetic sister clade to the Ascomycota and Basidiomycota, have left the most ancient fossils in the Rhynie Chert of Aberdeenshire in the north of Scotland (400 million years old). The Glomeromycota and several other fungi have been found associated with the preserved tissues of early vascular plants (Taylor *et al.* 2004a). Fossil spores from these shallow marine sediments from the Ordovician that closely resemble Glomeromycota spores and finely branched hyphae arbuscules within plant cells were clearly preserved in cells of stems of a 400 Ma primitive land plant, Aglaophyton, from Rhynie chert 455–460 Ma in age (Redecker *et al.* 2000; Remy *et al.* 1994) and from roots from the Triassic (250–199 Ma) (Berbee & Taylor 2010; Stubblefield *et al.* 1987).

Many other fungal preserved materials have been found and a very well-preserved Ascomycota fungal fossil (*Paleopyrenomycites devonicus*), consisting of perithecia immersed within stems of a Devonian plant (*Asteroxylon mackiei* Kidston and Lang), provides a minimum age for the Ascomycota and Basidiomycota at 452 Ma (Berbee & Taylor 2010; Taylor & Gaines 1999, 2004b, 2005). Basidiomycota is the sister group to the Ascomycota and the two phyla must be the same age. Basidiomycota are diagnosed by the hyphae with clamp connections and in modern ecosystems clamped hyphae permeate soil and organic matter. The oldest convincing basidiomycete fossils are of hyphal clamp connections from a Carboniferous coal ball (Pennsylvanian age, 299–318 Ma), which are much younger than even the minimum age of Ascomycota at 452 Ma (Berbee & Taylor 2010).

Fungi are an ancient group of organisms and their earliest fossils are from the Ordovician, 460–455 million years old (Redecker *et al.* 2000). Based on fossil evidence, the earliest vascular land plants appeared approximately 425 million years ago, and it is believed that fungi may have played an essential role in the colonization of land (Carris *et al.* 2012; Redeker *et al.* 2000). Mushroom structures preserved in amber from the Late Cretaceous (94 million years ago) are evidence that mushroom-forming fungi similar to those that exist today already existed when dinosaurs roamed the planet (Hibbett *et al.* 2003). However, the fungal fossil record is incomplete and provides only a minimum time estimate for when different groups of fungi evolved. Molecular data suggest that fungi are much older than indicated by the fossil record, and may have arisen more than 1 billion years ago, but the development of a mutually corroborating body of fossil and phylogenetic evidence is needed to clarify the evolution of organisms on Earth (Berbee & Taylor 2010; Carris *et al.* 2012; Parfrey *et al.* 2011).

Fungi were not fixed geographically but rather, fungal ranges changed more recently and dynamically through long-distance dispersal. The same geographical barriers affecting the spread of plants and animals also limited the historical spread of fungi. Fungi are not simply ancient and unchanging, but have evolved just as dynamically as any other group of eukaryotes (Berbee &

Taylor 2010).

The kingdom Fungi is one of the most diverse groups of organisms on Earth (Tedersoo *et al.* 2014). The fungi are a distinct group of organisms more closely related to animals than plants (FAO 2004). By their descent from an ancestor shared with animals about a billion years ago plus or minus 500 million years (Berbee & Taylor 2010), the fungi constitute a major eukaryotic lineage equal in numbers to animals and exceeding plants. The kingdom Fungi, distinct from plants and animals, became gradually accepted after Whittaker's classification (1969) (Abdel-Azeem 2010). Although the concept of the Fungi as one of the six kingdoms of life was introduced by Jahn & Jahn (1949) and a five kingdom system had been advanced by Whittaker (1959), neither of these works included a Latin diagnosis and the name was therefore invalid under the International Code of Botanical Nomenclature, until the required Latin description was provided by Moore in 1980 (Hibbet 2007). Presently, the extremely diverse group of organisms studied as "fungi" span three kingdoms, most belonging to the Fungi (*Eumycota*), while others are classified in the Protozoa and Chromista (*Straminipila*) (Abdel-Azeem 2010; Cavalier-Smith 1998; James *et al.* 2006). The word "fungi," lower case and not in italics, is commonly used as a collective term for organisms from all three kingdoms traditionally studied by mycologists (Abdel-Azeem 2010; Hawksworth 1991).

Estimates for the number of fungi in the world have been suggested by many authors and range up to ca. 13.5 million species (Adl *et al.* 2007; Blackwell 2011; Crous *et al.* 2006; Hawksworth 1991, 2001; Hawksworth & Kalin-Arroyo 1995; Hyde 1996; Hyde *et al.* 1997; Kirk *et al.* 2008; McNeely *et al.* 1990). It might be expected that the predicted numbers of fungi on Earth would have been considerably greater than the 1.5 million suggested by Hawksworth (1991), based on ratios of known fungi to plant species in regions where fungi were considered to be well studied, which is currently accepted as a working figure although recognized as conservative because numerous potential fungal habitats and localities remain understudied (Hawksworth 2001). This was based on a fungus to plant ratio of 6:1, in contrast to the much lower estimates suggested by Bisby and Ainsworth (1943) of 100 000 fungal species and by Martin (1951) of 250 000 species

based on one fungus for every phanerogam known at the time (Blackwell 2011). Analysis of environmental DNA samples from a soil community revealed a high rate of new species accumulation at the site, and these data supported an estimate of 3.5–5.1 million species according to O'Brien *et al.* (2005) and Blackwell (2011).

According to the present data, higher estimates of land plant numbers are slightly under 400 000 species (Joppa *et al.* 2010; Paton *et al.* 2008); fungal species numbers are expected to outnumber the land plant by 10.6:1 based on O'Brien *et al.* (2005). Higher ratios have even been predicted according to data from sequencing of clone libraries, although individual ecosystems will have variations. Fungi comprise some 100 000 described species, but the actual extent of global fungal diversity is estimated at 0.8 million to 5.1 million species according to data acquired from several molecular methods (Blackwell 2011; O'Brien *et al.* 2005; Taylor *et al.* 2010).

The Dictionary of the Fungi (Kirk *et al.* 2008) reported 98 998 species of all described fungi species (Figure 2.1) (excluding taxa treated under Chromista and Protozoa). The Dictionary estimated that known species has almost tripled in the period between the first edition in 1943 (38 000 described species) and 2008, amounting to an increase of more than 60 000 described species over the 65-year period (see Figure 2.1). Factors such as difficulty of isolation and failure to apply molecular methods may contribute to lower numbers of species in certain groups, but there cannot be any doubt that ascomycetes and basidiomycetes comprise the vast majority of fungal diversity (Figure 2.2) (Abdel-Azeem 2010; Blackwell 2011). Kirk *et al.* (2008) reported 1039 species as chromistan fungal analogues and 1165 as protozoan, in which 1038 are regarded as protozoan fungal analogues (Abdel-Azeem 2010).

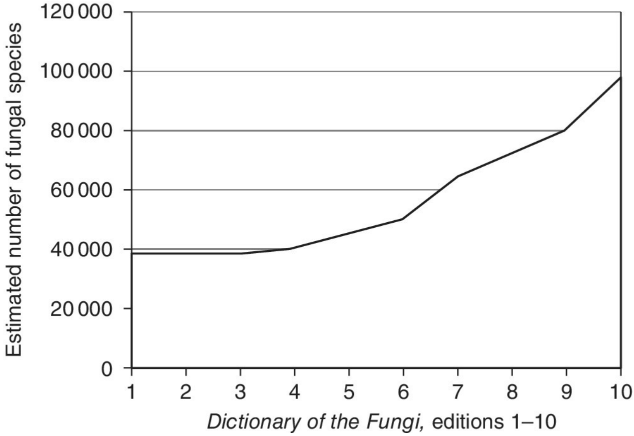

Figure 2.1 Numbers of known fungi from the *Dictionary of the Fungi* (editions 1–10, 1950–2008). Authors state that the large increase in species numbers in the 10th edition may be inflated because asexual and sexual forms were counted separately and molecular techniques that distinguish close taxa have been used.

Source: reproduced with permission from Blackwell (2011).

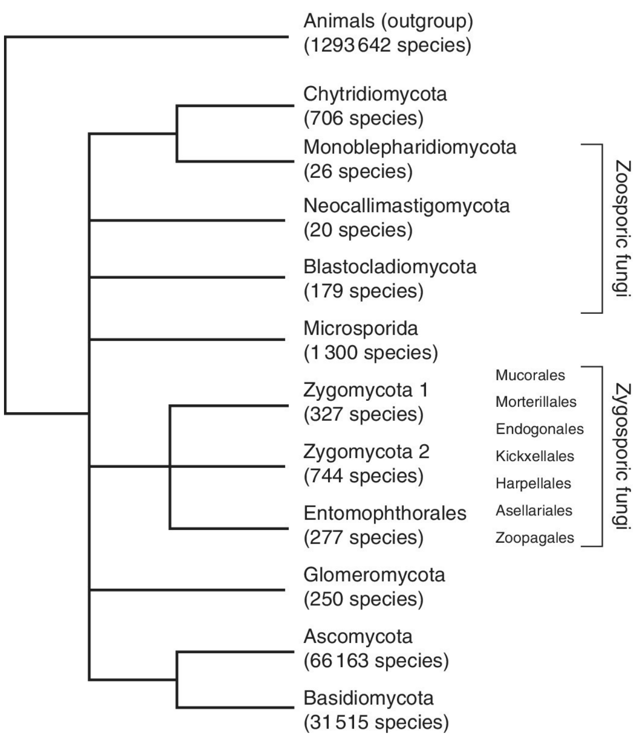

Figure 2.2 Fungal phyla and approximate number of species in each group (Kirk *et al.* 2008). Evidence from gene order conversion and multilocus sequencing indicates that microsporidians are Fungi (Lee *et al.* 2010). Zoosporic and zygosporic fungal groups are not supported as monophyletic. Tree based on Hibbett *et al.* (2007), White *et al.* (2006), and James *et al.* (2006).

Source: reproduced with permission from Blackwell (2011).

2.2 Ecological Diversity

Fungi are eukaryotic microorganisms consisting of fine threads known as hyphae, which together form a mycelium, or yeast forms; they play fundamental ecological roles as decomposers, mutualists, and pathogens of plants and animals. They obtain their nutrients in three basic ways, depending on dead and living material for their nutrition and growth: *saprobic*, if they grow on dead organic matter; *symbiotic*, when growing in association with other organisms; *parasitic*, when causing harm to another organism. They drive carbon cycling in forest soils, mediate mineral nutrition of plants, and alleviate carbon limitations of other soil organisms (Blackwell 2011; FAO 2004).

Saprobic fungi are those that feed on dead or decomposing organic matter. In the absence of chlorophyll to synthesize carbon compounds from the atmosphere's CO_2, such fungi secrete a number of enzymes which are able to decompose cellulose, hemicellulose, and lignin mainly from plants. Therefore, they have a mission of great ecological importance (Anguix 2011). They play a vital role in the life cycle of the biosphere, since all plant debris generated over time is mineralized and transformed into humus, thus recycling soil nutrients. This process involves the volatilization of carbon, hydrogen, and oxygen, and the release of nitrogen, phosphorus, potassium, sulfur, and many other elements. Saprobic fungi are provided with efficient enzyme complexes capable of degrading complex carbon sources such as cellulose, lignin or starch and transforming them into simple and nutritious molecules like sugars and amino acids. These enzymes show

different degrees of effectiveness in the degradation of substrates, determining the degree of specialization of these fungi. While some fungi exploit organic matter of any origin, others prefer more specific substrates. Thus we find humus decomposing fungi, coprophilous and lignicolous, among others according to the decomposing substrate (Anguix 2011; Fernández-Toirán *et al.* 2011a).

Concerning fruiting body production, several authors point out that the proportion of saprobes to total macrofungi is generally low (Vogt *et al.* 1992), although this depends on the amount of debris that accumulates in the forest. The volume and value of saprobic wild species used as food are small by comparison with the symbiotic edible fungi, though more edible saprobic species are collected.

Symbiotic fungi include lichenized fungi and mycorrhizas as the main forms of association. The first symbiotic associations with algae and cyanobacteria (Fernández-Toirán *et al.* 2011a) and about 20% of all fungi and 40% of the ascomycetes (13 500 species) are lichen-forming fungi (Lutzoni & Miadlikowska 2009). Lichens and lichenized fungi are estimated to comprise about 20 000 species (Feuerer & Hawksworth 2007).

Mycorrhizal fungi form symbiotic associations with plant roots, forming mycorrhizae, a term first used by Frank (1885) to define the mutually beneficial partnership between the hyphae of a fungus and the roots of a plant. This partnership has proven to be of great importance in forest ecosystems.

Mycorrhizae are the most common symbiotic fungi association because they occur in more than 90% of the plant species, including bryophytes and ferns (Pressel *et al.* 2010). They are often essential to their plant hosts because they take up water, nitrogen, phosphorus, and other nutrients from the soil and transfer them to the plant roots. Some of these fungi may not prosper or even grow without the host. Certain mycorrhizal fungi specialize in orchids and ericoid plants, and some are known to have invaded new habitats with successful invasive plants (Pringle *et al.* 2009).

There are two main types of mycorrhizal fungi associations: arbuscular mycorrhizae (AM) and ectomycorrhizae (ECM). AM

associations are more common and occur with up to 80% of all plant species and 92% of plant families. AM fungi are all included in the phylum Glomeromycota, a group with about 250 described species in a variety of taxa, though less diverse than ectomycorrhizal fungi (Blackwell 2011; Schüβler & Walker 2010; Schüβler *et al.* 2001; Wang & Qiu 2006).

More than 6000 species, mostly of mushroom-forming Basidiomycota, form ectomycorrhizae with about 10% of all plant families although their importance in the forestry world is enormous, as are trees and shrubs belonging to the families Pinaceae, Fagaceae, Betulaceae, and Salicaceae, among others (Fernández-Toirán *et al.* 2011b). Greater host specificity usually occurs in the ectomycorrhizal fungus–plant associations than in AM associations (Blackwell 2011; Smith & Read 2008).

A recent study has conservatively estimated global ectomycorrhizal fungal species richness at approximately 7750 species. However, on the basis of estimates of macromycete known and unknown diversity, a final estimate of ECM species richness would likely be between 20 000 and 25 000 (Rinaldi *et al.* 2008).

Moreover, ectomycorrhizae-forming fungi include many of the most common species, mainly from the divisions Basidiomycota (*Amanita* spp., *Boletus* spp., *Lactarius* spp., *Hebeloma* spp., etc.) and Ascomycota (*Tuber* spp., *Terfezia* spp., etc.) (Fernández-Toirán *et al.* 2011b). The fruiting bodies of some of these species, mushrooms, have great economic interest, being highly appreciated for human consumption, such as boletus, chanterelles, and truffles.

Parasitic fungi are characterized by living in different hosts (plant, animal or fungi) to which they cause more or less serious damage or even death. If causing disease in the host, they are considered pathogens. They are biotrophic when they need to live of living cells and necrotrophic when they degrade the dead host as a saprobic (Fernández-Toirán *et al.* 2011b).

Although some zoosporic and zygosporic fungi are plant pathogens, most plant pathogens are Ascomycota and Basidiomycota. A large number of Ascomycota and ca. 8000

species of Basidiomycota are plant pathogens (Blackwell 2011). Parasitic plant fungi play an important role in ecosystems, affecting competition between plant species and acting generally as balancing factors of the ecosystem. Thus, they can open holes in wood, creating microhabitats and favoring the establishment of other species, causing changes in the size and distribution of the plant population and increasing diversity. However, in monospecific forests and particularly in plantations of exotic species, fungi parasites can cause severe damage (Fernández-Toirán *et al.* 2011b).

Fungi have the ability to grow on and in both invertebrate and vertebrate animals. Many fungi can attack insects and nematodes; for example, they may play an important role in keeping populations of these animals under control. Insect-attacking fungi, called entomopathogens, include a wide range of fungi in phyla Ascomycota, Zygomycota, and Chytridiomycota (Carris *et al.* 2012).

There are relatively few fungal pathogens of vertebrates (only 200 – 300 species) but some of these fungi can have devastating impacts. Some examples are the frog killer, *Batrachochytrium dendrobatidis* Longcore, Pessier & D.K. Nichols, a member of phylum Chytridiomycota, that is the only chytrid known to parasitize a vertebrate animal (amphibians), and the Ascomycota *Geomyces destructans* Blehert & Gargas that causes “white-nose syndrome” in bats (Carris *et al.* 2012).

In humans, there are several different types of fungal infections, or mycoses. The most common are caused by dermatophytes, fungi that colonize dead keratinized tissue including skin, finger-, and toenails. Dermatophytes cause superficial infections such as ringworm that are unsightly and difficult to treat, but rarely serious. Some fungi are members of the resident microflora in healthy people, but become pathogenic in people with predisposing conditions, as, for example, *Candida* species. Another group of fungi are inhaled as spores and initiate infection through the lungs. These include *Coccidioides immitis* (coccidioidomycosis, commonly known as valley fever) and *Histoplasma capsulatum* (histoplasmosis) (Carris *et al.* 2012).

Parasitism can also occur between two fungi, such as *Hypomyces lateritius* that parasitize the hymenium of *Lactarius deliciosus* (L. ex Fr.) S.F.Gray, usually causing the disappearance of the lamellae. Another example is *Sepedonium chrysospermum* (Bull.) Fr. that parasitizes *Boletus edulis* Bull. Parasitism of some fungi on others suggests the existence of a natural biological control (Fernández-Toirán *et al.* 2011b).

Fungi grow in almost all habitats on Earth, surpassed only by bacteria in their ability to withstand extremes in temperature, water activity, and carbon source (Raspor & Zupan 2006). Tropical regions of the world are considered to have the highest diversity for most groups of organisms (Hillebrand 2004), and this is generally true for fungi as well (Arnold & Lutzoni 2007).

In temperate deserts mycorrhizal boletes, agarics, and rust and smut fungi are common. A surprising number of wood-decaying basidiomycetes have been discovered on living and dead desert plants, including cacti (Blackwell 2011).

Fungi also grow at very low temperatures as can be observed on the deterioration of historic shelters built by Antarctic explorers. Although there are not large numbers of species, it is important to consider this fungal habitat in diversity studies (Blackwell 2011; Held *et al.* 2005). In Arctic and Antarctic regions, lichens have often been reported (Wirtz *et al.* 2008), and yeasts are active under frozen conditions in the Antarctic (Amato *et al.* 2009; Vishniac 2006). In some cases, yeasts isolated from the Antarctic (based on 28S rDNA barcoding) have been reported from varied habitats, including human infections, the gut of insects, deep seas, and hydrocarbon seeps (Kurtzman & Fell 1998). Although some fungi are specialized for cold regions, others simply occupy a wide variety of environmental conditions (Blackwell 2011).

Many regions and habitats of the world need to be included in fungal diversity studies, including the following (Blackwell 2011).

2.2.1 Freshwater Fungi

More than 3000 species of ascomycetes are specialized for a saprobic lifestyle in freshwater habitats where they have enhanced

growth and sporulation (Kirk *et al.* 2008; Shearer & Raja 2010; Shearer *et al.* 2007). Other fungi are present in water, and some of these are active in degrading leaves in streams. A few specialized freshwater basidiomycetes are also known. Flagellated fungi occur in aquatic habitats, including Chytridiomycota, Blastocladiomycota, and Monoblepharomycota (James *et al.* 2006). *Batrachochytrium dendrobatidis*, the recently described amphibian killer, is an aquatic chytrid (Longcore *et al.* 1999).

2.2.2 Marine Fungi

According to estimates performed by Hyde *et al.* (1998), 1500 species of marine fungi occur in a wide range of taxonomic groups. Many of these fungi are distinct from freshwater aquatic species, and they may be saprobic on aquatic plant substrates. Some species have characteristics such as sticky spore appendages, indicators of specialization for the marine habitat (Kohlmeyer *et al.* 2000). Most marine fungi are ascomycetes and basidiomycetes, including ascomycete and basidiomycete yeasts (Nagahama 2006). Some of the yeasts degrade hydrocarbon compounds present in natural underwater seeps and spills (Davies & Westlake 1979). Certain ascomycetes are specialized on calcareous substrates, including mollusk shells and cnidarian reefs. Even a few mushroom-forming basidiomycetes are restricted to marine waters (Binder *et al.* 2006). Some fungi use other marine invertebrates as hosts (Kim & Harvell 2004), including antibiotic producers that live in sponges (Bhadury *et al.* 2006; Pivkin *et al.* 2006; Wang *et al.* 2008). A wide variety of fungi considered to be terrestrial are also found in marine environments (Kurtzman & Fell 1998; Morris *et al.* 2011; Murdoch *et al.* 2008).

2.2.3 Endophytes of Plant Leaves and Stems

Most plants on Earth are infected with fungi endophytes, that do not cause disease symptoms (Saikkonen *et al.* 1998). Endophytes

from a broad array of taxonomic groups occur between the cells of above-ground plant parts (Arnold 2007; Rodriguez *et al.* 2009). Some grass endophyte species produce alkaloid toxins effective against insects, other invertebrate animals, and vertebrates (Clay *et al.* 1993). Some grass endophytes are transmitted to the host offspring in seeds, and others inhibit sexual reproduction in the host and are dispersed within plant parts such as leaf fragments. For grass endophytes that reproduce sexually, fertilization may occur by insect dispersal. Infected hosts have increased water intake and these plants often have increased growth compared to uninfected hosts.

A much more diverse group of endophytic fungi are associated with a variety of dicots and conifers (Rodriguez *et al.* 2009), many from the ascomycetes group. In tropical habitats, plant leaves can acquire multiple infections as they mature, and there is strong evidence that the endophytes protect leaves of plants from infection when they were challenged with pathogens (Arnold *et al.* 2003). Vega and colleagues (2010) also found high diversity of endophytes in cultivated coffee plants. Interestingly, some of these were insect pathogens and experiments are being conducted to develop endophytes as biological control agents of insect pests.

2.2.4 Fungi from Arthropod and Invertebrate Animals

Arthropod and insect-associated fungi are poorly studied (Hawksworth 1991; Mueller & Schmit 2007; Rossman 1994; Schmit & Mueller 2007) but estimates of insect-associated fungi suggest the existence of 20 000–50 000 species (Rossman 1994; Schmit & Mueller 2007; Weir & Hammond 1997a,b). Insects may be food for fungi, especially in low nitrogen environments. Studies of the ectomycorrhizal basidiomycete *Laccaria bicolor* (Maire) P.D.Orton led to the surprise discovery that the fungus was not insect food but rather, the fungus and the host tree benefited by obtaining substantial amounts of nitrogen from the insects (Klironomos & Hart 2001). The predatory habit has arisen independently on several occasions in at least four phyla of fungi and oomycetes. Predatory fungi such as *Arthrobotrys* and

Dactylella trap, capture, or control nematodes and other small invertebrate animals in soils and wood (Barron 1977). Global estimates of arthropods were revised from 30 million to 5–10 million (Ødegaard 2000) and although not all insects and arthropods associate with fungi, the numbers of insect-associated fungi must be very high (Blackwell 2011).

2.3 Global Diversity of Soil Fungi

Fungi are broadly distributed in all terrestrial ecosystems and play major roles in ecosystem processes (soil carbon cycling, plant nutrition, pathology), but the distribution of species, phyla, and functional groups as well as the determinants of fungal diversity and biogeographic patterns are still poorly understood (Tedersoo *et al.* 2014).

The latitudinal gradient of diversity is a highly general spatial pattern of diversity with very few notable exceptions (Hillebrand 2004). At a global scale, the biomass and relative proportions of microbial groups vary with the concentration of growth-limiting nutrients in soils and plant tissues. The distribution of microbes may reflect latitudinal variation in ecosystem nutrient dynamics (Fierer *et al.* 2009; Serna-Chavez *et al.* 2013; Tedersoo *et al.* 2014; Xu *et al.* 2013). Richness of nearly all terrestrial and marine macroorganisms is negatively related to increasing latitude (Hillebrand 2004) as a result of the combined effects of climate, niche conservatism, and rates of evolutionary radiation and extinction (Mittelbach *et al.* 2007; Tedersoo *et al.* 2014).

Despite the enormous diversity and importance of fungi in ecosystem function, their general diversity patterns or functional roles over large geographic scales are poorly understood. Tedersoo *et al.* (2014) used a global dataset to unravel the roles of climatic, edaphic, floristic, and spatial variables governing global-scale patterns of soil fungal diversity. They also showed that fungi largely exhibit strong biogeographic patterns that appear to be driven by dispersal limitation and climate (Tedersoo *et al.* 2014).

The microscopic size and hidden existence of most below-ground

organisms limit the knowledge of their global ecology; however, molecular techniques for analyzing soil communities have provided unprecedented opportunities for understanding soil biodiversity and testing whether global diversity patterns established for above-ground biota also apply to soil biota. Tedersoo *et al.* (2014) characterized fungal communities in soil samples from 365 separate locations worldwide (including all continents except Antarctica), all of which were sampled, processed, and analyzed by the same methods (Wardle & Lindahl 2014).

At a global scale, mean annual precipitation seemed to be the strongest driver of the richness of fungal operational taxonomic units but soil properties, and particularly soil pH and calcium concentration, also had important positive effects. Soil fungi are generally considered as acidophiles when compared to bacteria but the current results suggest that, rather than a preference for acidic conditions, they have a wider range of pH tolerance (Tedersoo *et al.* 2014; Wardle & Lindahl 2014).

The relative richness of the main functional fungi groups, ectomycorrhizae, saprotrophs, and pathogens, provides a wide variation among the major earth biomes, consistent with the separate set of factors affecting each group. Ectomycorrhizal fungal richness is most strongly related to the richness of host plant species and high soil pH; saprotroph richness is positively related to mean annual precipitation; and pathogen richness is negatively related to latitude but positively related to nitrogen availability (Tedersoo *et al.* 2014; Wardle & Lindahl 2014).

Total fungal richness increases toward the equator, in line with the general pattern of decline of species richness with increasing latitude (Hillebrand 2004; Taylor & Gaines 1999), but major groups of fungi defy this pattern. Ectomycorrhizal fungal richness is greatest at mid- to high northern latitudes (coinciding with temperate and boreal forest), and richness within several ascomycete groups (notably the Leotiomycetes, which include fungi that form mycorrhizal associations with ericoid dwarf shrubs) increases toward the poles. Globally, fungal richness does not decline as sharply as plant species diversity with increasing latitude; the result is that the ratio of fungal to plant richness rises

exponentially toward the poles. Fungi are thus a key component of total terrestrial biodiversity at high latitudes, with important implications for conservation. Reliable estimates of this ratio are important for deriving global fungal diversity from measures of plant diversity.

According to Tedersoo *et al.* (2014), at a global scale the best predictors of fungal richness and community composition are climatic factors, followed by edaphic and spatial variables. Richness of all fungi and functional groups is causally unrelated to plant diversity, ectomycorrhizal root symbionts being the exception. They emphasize that plant-to-fungi richness ratios decline exponentially toward the poles, and that predictions assuming globally constant ratios can overestimate fungal richness by 1.5–2.5-fold. Similar biogeographic patterns were found for fungi, plants and animals, with the exception of several major taxonomic and functional groups that run counter to overall patterns. Fungi exhibited strong biogeographic links among distant continents, revealing a relatively efficient long-distance dispersal compared with macroorganisms (Tedersoo *et al.* 2014).

2.4 Wild Edible Fungi

Wild edible mushrooms have been collected and consumed by people for thousands of years. Since time immemorial, a considerable number of identified species of fungi have made a significant contribution to human food and medicine.

The use of edible species by people living in Chile 13 000 years ago is documented in archaeological records (Rojas & Mansur 1995). China has a history of consumption and use of wild mushrooms that was first reliably noticed several hundred years before Christ (Aaronson 2000). Edible mushrooms were gathered in the forests during Greek and Roman antiquity, but were appreciated mainly by people of higher status (Buller 1914). The Roman Empire is well known for the mushroom consumption of its emperors, who employed food tasters to ensure that the mushrooms were safe to eat (Jordan 2006). The Caesar mushroom (*Amanita caesarea* (Scop.) Pers.) refers to an ancient Italian

tradition that still exists in many parts of Italy, using a diversity of edible species dominated today by truffles (*Tuber* spp.) and porcini (*Boletus edulis*). In China, many wild mushroom species have been valued for centuries, not only for food but also for their medicinal properties. These values and traditions are still highly relevant today and are confirmed by the wide range of wild mushrooms picked from the forests and fields. China also leads the exports of cultivated mushrooms (FAO 2004; FAOStat 2015).

The tradition of wild edible mushroom use exists from ancient times in many countries. Although less well known, countries like Mexico and Turkey and vast areas of Central and Southern Africa also have a long and important tradition of edible wild mushrooms. The list of countries where wild mushrooms are consumed and provide earnings to rural people is very long and widespread around the world (Table 2.1) (FAO 2004).

Table 2.1 Country records of wild useful fungi (edible, medicinal, and other uses).

Country	**No. of species**		**Reference**
Afghanistan	2	Edible (2)	Batra 1983; Sabra & Walter 2001
Algeria	3	Edible (3)	Alsheikh & Trappe 1983; Kytovuori 1989
Angola	2	Edible (2)	Rammeloo & Walleyn 1993
Argentina	5	Food (5)	Deschamps 2002; Gamundi & Horak 1995
Armenia	15	–	Nanaguylan 2002 personal communication according to

			FAO 2004
Australia	16	Food (7) Edible (1) Medicinal (5) Dye (1) Tinder (1) Cosmetic (1) Not known (3) Other (2)	Kalotas 1997
Belarus	14	Edible (14)	Malyi 1987
Benin	93	Food (90) Edible (1) Medicinal (2)	Antonin & Fraiture 1998; de Kesel *et al.* 2002; Walleyn & Rammeloo 1994; Yorou & de Kesel 2002; Yorou *et al.* 2002
Bhutan	13	Edible (12) Food (1)	Namgyel 2000
Bolivia	1	Food (1)	Boa 2002, personal communication
Botswana	3	Edible (2) Food (1)	Rammeloo & Walleyn 1993; Taylor *et al.* 1995
Brazil	30	Food (29) Medicinal (1)	Prance 1984; www.agaricus.net
Bulgaria	213	Edible (114) Not known (93) Not eaten (1)	Iordanov *et al.* 1978
Burkina Faso	2	Edible (2)	Rammeloo & Walleyn 1993
Burundi	31	Edible (31)	Buyck 1994;

			Walleyn & Rammeloo 1994
Cameroon	6	Edible (6)	Pegler & Vanhaecke 1994; Rammeloo & Walleyn 1993
Canada	46	Edible (16) Food (19) Medicinal (11) Tinder (2)	Marles *et al.* 2000; Tedder *et al.* 2002; www.for.gov.bc.ca
Central African Republic	14	Edible (10) Medicinal (3) Other – string (1)	Rammeloo & Walleyn 1993; Walleyn & Rammeloo 1994
Chile	24	Food (8) Edible (16) Medicinal (1)	FAO 1998; Minter *et al.* 1987; Schmeda-Hirschmann *et al.* 1999
China	220	Medicinal (19) Food (10) Not edible (2) Edible (186) Not known (2)	Birks 1991; Cao 1991; Chamberlain 1996; Dong & Shen 1993; Gong & Peng 1993; Hall *et al.* 1998; Härkönen 2002; He 1991; Huang 1989; Li 1994; Liu 1990; Liu & Yang 1982;

			Guozhong 2002, personal communication; Pegler &Vanhaecke 1994; Tu 1987; Winkler 2002; www.zeri.org; Xiang & Han, 1987; Yang 1990, 1992; Yang & Yang 1992; Zang 1984, 1988; Zang & Petersen 1990; Zang & Pu 1992; Zang & Yang 1991; Zhuang 1993; Zhuang & Wang 1992
Congo (Democratic Republic)	110	Medicinal (2) Edible (107) Other – jewelry (1)	Degreef *et al.* 1997; Pegler & Vanhaecke 1994; Rammeloo & Walleyn 1993; Walleyn & Rammeloo 1994
Congo (Republic)	6	Edible (6)	Rammeloo & Walleyn 1993
Costa Rica	59	Hallucinogen (5) Poisonous (1)	Saenz *et al.* 1983

		Edible (60)	
Cote d'Ivoire	4	Edible (3) Food (1)	Ducousso *et al.* 2002; Locquin 1954; Pegler & Vanhaecke 1994; Rammeloo & Walleyn 1993
Egypt	3	Edible (3)	Zakhary *et al.* 1983
Ethiopia	2	Edible (2)	Tuno 2001
Fiji	1	Food (1)	Markham 1998
Gabon	5	Edible (2) Medicinal (2) Other – string (1)	Rammeloo & Walleyn 1993; Walleyn & Rammeloo 1994, Note: another 15+ types are listed in Walker 1931, by local name only
Ghana	17	Edible (12) Medicinal (6) Food (1)	Ducousso *et al.* 2002; Obodai & Apetorgbor 2001; Rammeloo & Walleyn 1993; Walleyn & Rammeloo 1994
Guatemala	38	Food (38)	Flores *et al.* 2002, personal communication
Guinea	1	Edible (1)	Walleyn &

			Rammeloo 1994
Guyana	1	Edible (1)	Simmons *et al.* 2002
Hong Kong Special Administrative Region, China	251	Edible (189) Medicinal (113)	Chang & Mao 1995
India	83	Edible (64) Medicinal (8) Other – spice (2) Other – perfume (1) Food (6)	Birks 1991; Boruah *et al.* 1996; Singh & Rawat 2000; Harsh *et al.* 1996; Pegler &Vanhaecke 1994; Purkayastha & Chandra 1985; Richardson 1991; Sarkar *et al.* 1988; Sharda *et al.* 1997; Sharma & Doshi 1996
Indonesia	7	Food (6) Medicinal (1) Edible (1)	Burkhill 1935; Ducousso *et al.* 2002
Iraq	3	Edible (3) Food (1)	Al-Naama *et al.* 1988; Alsheikh & Trappe 1983
Israel	3	Edible (3)	Wasser 1995
Jordan	9	Food (7) Edible (2)	Ereifej & Al-Raddad 2000; Sabra & Walter 2001
Kenya	11	Edible (5)	Pegler &

		Medicinal (2) Other – dye (2) Hallucinogen (2) Poisonous (1)	Vanhaecke 1994; Rammeloo & Walleyn 1993; Walleyn & Rammeloo 1994
Korea	1	Edible (1)	Wang *et al.* 1997
Kuwait	2	Edible (1) Food (1) Medicinal (1)	Alsheikh & Trappe 1983
Kyrgyzstan	32	Edible (32)	EI'chibaev 1964
Laos	28	Edible (19) Food (5) Medicinal (3) Other (1)	Hosaka 2002, personal communication; http// giechgroup.hp.infoseek.co kinoko/ eng.html
Lesotho	1	Edible (1)	Rammeloo & Walleyn 1993
Libyan Arab Jamahiriya	2	Edible (2)	Alsheikh & Trappe 1983
Madagascar	75	Edible (72) Food (1) Medicinal (1) Other – dye (1)	Bouriquet 1970; Ducousso *et al.* 2002; Rammeloo & Walleyn 1993; Richardson 1991; Walleyn & Rammeloo 1994
Malawi	76	Edible (75) Medicinal (1)	Rammeloo & Walleyn 1993;

		Hallucinogen (1) Poisonous (1) Insecticidal (1)	Walleyn & Rammeloo 1994; see also www.malawifungi.org
Malaysia	7	Edible (6) Food (1)	Burkhill 1935; Pegler & Vanhaecke 1994
Mauritius	5	Edible (5)	Rammeloo & Walleyn 1993; Walleyn & Rammeloo 1994
Mexico	307	Edible (119) Food (180) Medicinal (16) Insecticidal (2) Hallucinogen (1) Other – dye (1)	Lopez *et al.* 1992; Mata 1987; Montoya-Esquivel 1998; Montoya-Esquivel *et al.* 2001; Moreno-Fuentes *et al.* 1996; Richardson 1991; Villarreal & Perez-Moreno 1989; www.semarnat.gob.mx; Zamora-Martinez *et al.* 2000; Zamora-Martinez *et al.* 1994
Mozambique	22	Food (22)	Uaciquete *et al.* 1996; Wilson *et al.*

			1989
Morocco	12	Edible (10) Other – perfume (2)	Alsheikh & Trappe 1983; Kytovuori 1989; Moreno-Arroyo *et al.* 2001; Richardson 1991; FAO 2001
Myanmar	1	Edible (1)	Pegler & Vanhaecke 1994
Namibia	4	Edible (2) Medicinal (1) Cosmetic (1) Food (1)	Rammeloo & Walleyn 1993; Taylor *et al.* 1995; Walleyn & Rammeloo 1994
Nepal	98	Edible (41) Medicinal (8) Food (32) Other – perfume (1)	Adhikari 1999; Adhikari & Durrieu 1996; Richardson 1991; Zang & Doi 1995
Nigeria	23	Edible (4) Food (16) Medicinal (6) Cosmetic (1) Poisonous (1) Animal poison (1)	Alofe *et al.* 1996; Oso 1975; Rammeloo & Walleyn 1993; Walleyn & Rammeloo 1994
Pakistan	21	Edible (21)	Batra 1983; Gardezi 1993; FAO 1993; Pegler &

			Vanhaecke 1994; Syed-Riaz & Mahmood-Khan 1999
Papua New Guinea	36	Edible (26) Not eaten (8) Other – raw material (1)	Sillitoe 1995
Peru	16	Edible (15) Food (1)	Diez 2003, personal communication: Collecting *Boletus edulis* Bull. for commercial purposes in Peru; Remotti & Colan 1990
Philippines	7	Edible (3) Food (4)	Novellino 1999; Pegler & Vanhaecke 1994
Poland	14	Food (14)	www.grzyby.pl
Réunion	1	Edible (1)	Rammeloo & Walleyn 1993
Russian Federation	240	Edible (226) Poisonous (1) Not known (7) Medicinal (3) Not Edible (7)	Saar 1991; Vasil'eva, 1978; Note: This is only for the Russian far east
Saudi Arabia	3	Edible (3) Food (1)	Alsheikh & Trappe 1983; Bokhary & Parvez 1993; Kirk *et al.*

			2001
Senegal	13	Edible (10) Food (2) Medicinal (1)	Ducousso *et al.* 2002 ; Thoen & Ba 1989
Sierra Leone	1	Edible (1)	Pegler & Vanhaecke 1994
Singapore	1	Food (1)	Burkhill 1935
Slovenia	23	Edible (22) Not Edible (1)	www.matkurja.com
Somalia	2	Edible (2)	Rammeloo & Walleyn 1993
South Africa	11	Edible (9) Hallucinogen (2) Poisonous (1)	Pegler & Vanhaecke 1994; Walleyn & Rammeloo 1994
Spain	61	Food (61)	Cervera & Colinas 1997; Martinez *et al.* 1997
Sri Lanka	2	Edible (2)	Pegler & Vanhaecke 1994
Tanzania	48	Edible (40) Food (5) Medicinal (4) Not Eaten (1)	Härkönen *et al.* 1994a, 1994b; Rammeloo & Walleyn 1993; Walleyn & Rammeloo 1994
Thailand	20	Food (20)	Jones *et al.* 1994; Pegler & Vanhaecke 1994; Stamets

			2000
Turkey	49	Edible (30) Food (19)	Afyon 1997; Caglarirmak *et al.* 2002; Demirbas 2000; Sabra & Walter 2001; http// www.ogm.gov.tri; Yilmaz *et al.* 1997
Uganda	10	Edible (10)	Katende *et al.* 1999; Pegler & Vanhaecke 1994;
Ukraine	160	Edible (160)	Zerova & Rozhenko 1988
Uruguay	7	Food (7)	Deschamps 2002
United States of America	83	Edible (71) Medicinal (11) Food (1)	Birks 1991; Lincoff & Mitchel 1977; Singer 1953; www.mykoweb.com
Vietnam	1	Food (1)	Burkhill 1935
Yugoslavia (now Serbia And Montenegro)	4	Food (3) Other – perfume (1)	Richardson 1988; Zaklina 1998
Zambia	23	Edible (4) Food (18) Medicinal (1)	Pegler & Piearce 1980; Piearce 1981; Rammeloo & Walleyn 1993; Walleyn & Rammeloo

			1994
Zimbabwe	12	Food (12)	Boa *et al.* 2000

Adapted from Annex 2, FAO (2004).

The list of wild useful fungi (edible, medicinal and other uses) (see Table 2.1) includes over 2800 records from 85 countries and was prepared from a preliminary database record of published information. The mycological literature is extensive in many developed countries but often there is no clear indication of which species are eaten as food. Only uses of practical or economic importance have been included; ceremonial or religious uses are omitted. In Table 2.1 are shown the total number of useful species and the main number of species with each of the uses (edible, food, medicinal, cosmetic and other, such as tinder, jewellery, spice, perfume, etc.) in each of the 85 countries. For details on the names of the species used in each country, Annex 2 of FAO (2004) can be consulted.

Mushrooms can make a substantial contribution to the diet of poor people in developing countries but they can also be an important source of income. The list of countries where wild fungi are reported to be consumed and provide income to rural people is impressive. Wild edible fungi are sold in many local markets and commercial harvesting has provided new sources of income for many rural people (Arora 2008; FAO 2004).

2.4.1 Diversity of Wild Edible Mushrooms

Edible mushrooms are the fleshy and edible fruit bodies of several species of macrofungi (fungi that produce visible fruiting structures – mushrooms, carpophores or sporophores). They can appear either below ground (hypogeous) or above ground (epigeous) where they may be picked by hand (Chang & Miles 1989). Edibility may be defined by criteria that include absence of poisonous effects on humans and desirable taste and aroma (Arora 1986; Rubel & Arora 2008). Wild edible fungi are important for three main reasons:

- as a source of food (plus health benefits)
- as a source of income

• to maintain the health of forests (FAO 2004).

There are more than 200 genera of macrofungi which contain species of use to people, mostly because of their edible properties. The FAO (2004) makes a clear distinction between edible mushrooms and those that are consumed as food, since including all edible species as "food" would greatly outnumber the species consumed by people around the world (Table 2.2). A total of 1154 edible and food species was recorded, from the total 2327 wild useful species compiled from 85 countries (see Table 2.2). The number of species eaten is sometimes only a fraction of those available. The species eaten in one country or region often differ from neighboring areas and in some cases there are dramatic changes in consumption tradition. The tradition of eating wild edible fungi goes from Mexico (180 species) to west Guatemala (38 species) then is absent from much of Honduras and Nicaragua, even though both contain forest areas that in theory support production of edible fungi.

Table 2.2 Numbers of species of wild edible and medicinal fungi (FAO 2004).

Category	No. of species	Percentage of total
1 Edible only	1009	43
2 Edible and medicinal	88	4
3 Food only	820	35
4 Food and medicinal	249	11
5 Medicinal only	133	6
6 Other uses (none of above)	29	1
TOTAL wild useful species	**2327**	–
ALL edible only (1 + 2)	1097	–
ALL food (3 + 4)	1069	–
ALL medicinal (2 + 4 + 5)	470	–

Note: Compiled from more than 200 different sources from 110 countries,

but excludes a detailed review of species from developed countries. Varieties and subspecies are counted separately. The categories “food” and “edible” are mutually exclusive. To distinguish clearly between use and properties of a species, substantial numbers of edible species lack confirmed use as food.

The reasons for these different patterns of use are not always clear but there is a tendency of less frequent use as people move away from the land (FAO 2004; Rubel & Arora 2008). Rural people in Guatemala have a positive and informed attitude of eating wild fungi which people living in cities lack (Lowy 1974). In Malawi, educated people living in towns have lost the strong local traditions that rural communities maintain and have even acquired a suspicious approach towards wild fungi (Lowore & Boa 2001).

According to the FAO (2004) and Rubel and Arora (2008), the poorer the people, the more likely they are to use wild edible fungi. Some traditions are lost as people become better educated and live away from the land and they show an increasing reluctance to eat all but the most common species (Lowy 1974). In Korea, China, the Russian Federation, and Japan, the tradition of eating wild edible fungi is much stronger and seems to have resisted the changes experienced elsewhere (FAO 2004).

Many macrofungi are not worth eating even when they are not toxic. Others are simply inedible, lacking one or more of the above-described characteristics. In comparison, the number of toxic or poisonous species is very small, and just a very few are mortal. However, this very small group of lethal species has significantly influenced attitudes to eating wild fungi, creating mycophobic behavior and potential barriers to wider marketing in many places.

Before assuming that any wild mushroom is edible, it should be exactly identified. Accurate determination and proper identification of a species is the only safe way to ensure edibility, and the only protection against possible accidents. Some mushrooms that are edible for most people can cause allergic reactions in some individuals, and old or improperly stored specimens can cause food poisoning.

The risk associated with poisonous and lethal species is often exaggerated since occurrences of poisoning and deaths are few

when compared to the regular and safe consumption of edible species. Publicity, cultural attitudes, and the increasing urban, nature-ignorant population continue to propagate an intrinsic fear of wild fungi in some societies (FAO 2004, 2009; Rubel & Arora 2008). This is more commonly found in developed countries and has undoubtedly led to general beliefs that global use of wild edible fungi is small-scale and restricted to key areas, which is not true, as conclusively shown in FAO (2004) (see Table 2.1). The patterns of use of wild edible fungi are both extensive and intensive, though they do vary.

In addition to those different patterns of use, edibility is a feature that can generate conflicting reports in literature and in field guides. Some species are recommended as edible in some literature and rejected as poisonous in others (FAO 2004; Rubel & Arora 2008). One of the cases of contradictory concepts about edibility is the false morel, *Gyromitra esculenta* (Pers. ex Pers.) Fr., that people from eastern Finland consider a delicacy after precooking, while guides in the United States and elsewhere consider that is poisonous and should not be eaten (FAO 2004). Some appropriate processing methods may render edible certain mushrooms reported as toxic, “poisonous” or not edible in mushroom field guides. For example, *Boletus luridus* Schaeff., *Boletus erythropus* Pers, and their close relatives are commonly eaten in China and Europe (especially Italy); *Boletus satanas* Lenz is eaten in Sicily after a complex cooking process; *Boletus subvelutipes* Peck is eaten in Japan and has been safely served for years by restaurants in Massachusetts; *Gomphus floccosus* Schw. (Singer) is commonly sold in the markets of Mexico and China; acrid, red-capped russulas such as *Russula emetica* (Schaeff.) Pers. are widely eaten after being cooked or salted; various peppery species of *Lactarius* such as *Lactarius torminosus* (Schaeff.) Pers. form an important part of the cuisine of northern European, Russia, and Siberia (Rubel & Arora 2008).

Traditional knowledge is increasingly reported, as in the case of Korean communities which include 158 practices within 22 families, 33 genera, and 38 species of mushrooms, with Tricholomataceae (23.20%), Pleurotaceae (13.10%), Polyporaceae (8.21%), and Hymenochaetaceae (6.33%) as the most

representative families. The results revealed 24 modes of preparation for the mushrooms, with the most common methods being seasoned cooked mushrooms (40.75%), soups (13.84%), teas (12.18%), simmered (9.19%), and roasted (6.20%) (Kim & Song 2014).

The major genera of wild edible fungi are described in Table 2.3, with brief notes on medicinal species. The wild edible fungi can be divided into two categories: those containing species that are broadly consumed and often exported in significant quantities, such as the genus *Boletus* and *Cantharellus*, and those with species that are eaten usually in small amounts and rarely exported (FAO 2004).

Table 2.3 Important genera of wild fungi with notes on uses and trade (FAO 2004).

Genus	No. of species, use and properties	Country use and general notes
Agaricus	**60** Food 43 Edible 17 Medicinal 6	Edible species reported from 29 countries, as food in 13 (underreported, though note possible confusion between wild and cultivated origins). *Agaricus* species are regularly collected from the wild but only cultivated forms are exported. Some species are poisonous. *A. bisporus* (J.E. Lange) Emil J. Imbach is the most commonly

		cultivated edible fungus. The medicinal *A. blazei* Murrill (1945) ss. Heinem. is exported from Brazil to Japan and cultivated and sold in China
Amanita	**83** Food 42 Edible 39 Medicinal 7	Edible species reported from 31 countries; as food in 15 (underreported). *Amanita caesarea* (Scop.) Pers. is highly valued in Mexico, Turkey, and Nepal. Few species are traded across national borders. There are a notable number of poisonous species. *Amanita phalloides* (Vaill. ex Fr.) Link is a major cause of deaths around the world from consumption of wild fungi
Auricularia	**13** Food 10 Edible 3 Medicinal 4	Edible species reported from 24 countries, as food in 10 (underreported). A global genus with a relatively small number of species. Known generically

		as "ear fungi," they are distinctive, easily recognized and consumed by forest dwellers in Kalimantan as well as rural communities in all continents. Some species have medicinal properties. There is a major trade in cultivated species though few data have been seen. Key species: *A. auricula-judae* (Bull.) J. Schröt.
Boletus	**72** Food 39 Edible 33 Medicinal 7	Edible species reported from 30 countries; as food in 15 (underreported). *Boletus edulis* Bull. is the best known species, regularly collected and sold, and a major export from outside and within Europe. There are some poisonous species but few incidents. "Bolete" is a general description of a macrofungus with a stalk and

		pores on the underside of the cap. Apprehension exists about eating "boletes" in east and southern Africa
Cantharellus	**42** Food 22 Edible 20 Medicinal 3	Edible species reported from 45 countries; as food in 22 (underreported). A diverse and cosmopolitan genus containing widespread species such as *C. cibarius* Fr. Sold in markets in many countries, sometimes in functional mixtures of different species. Major quantities are collected and exported around the world. No poisonous species
Cordyceps	**37** Edible ?35 Medicinal 9	Useful species (mostly medicinal) reported from three countries. The only reason for eating species is for health benefits. Collected intensively in parts of China and less so in Nepal. Many species described

		from Japan, but local use uncertain. Widely valued for its medicinal properties and an important source of income for collectors. Key species: probably *C. sinensis* (Berk.) Sacc.and *C. militaris* (L.) Fr.
Cortinarius	**50** Food 30 Edible 20 Medicinal 10	Edible species reported from 11 countries; as food in three. Widely disregarded in Europe and North America because of concern about poisonous species. Most records of local use are restricted to a few countries, e.g. China, Japan, the Russian Federation, and Ukraine. No known export trade
Laccaria	**14** Food 9 Edible 5 Medicinal 4	Edible species reported from 17 countries; as food in four (underreported). Regularly collected and eaten, also sold widely in markets.

		No reports of export trade, which is unsurprising given their generally small size and unremarkable taste. Key species is *L. laccata* (Scop.) Cooke
Lactarius	**94** Food 56 Edible 38 Medicinal 7	Edible species reported from 39 countries; as food in 17 (underreported). Many different species are regularly collected and eaten. Key species such as *L. deliciosus* (L. ex Fr.) S.F. Gray are highly esteemed and there is a valuable trade in Europe. Several key species frequently sold in local markets. Little reported export activity despite widespread popularity, perhaps reflecting the diversity of species on offer
Leccinum	**22** Food 4 Edible 9	Edible species reported from eight countries; as food in two. Widely eaten and

		collected but little trade beyond national boundaries. Key species *L. scabrum* (Bull.) Gray. Possible exports from pine plantations in tropics, but poorly understood
Lentinula	**3** Food 2 Edible 1 Medicinal 1	Edible species reported from six countries; as food in four. *Lentinula edodes* (Berk.) Pegler is the key species (= *Lentinus edodes*). Known as shiitake, it is cultivated in many countries and is an important commercial species (nearing 30% cultivated amount). Cultivated shiitake is exported
Lentinus	**28** Food 16 Edible 12 Medicinal 5	Edible species reported from 24 countries; as food in eight (underreported). Although many different species are collected and used locally, only two or three are of any

		significance. Key species probably *L. tuber-regium* (Fr.) Fr., valued for its medicinal properties. Little or no no export trade
Lycoperdon	**22** Food 9 Edible 10 Medicinal 10	Edible species reported from 19 countries; as food in seven (underreported). There are many records of species being eaten but typically reports are of small-scale collecting and use. Only market sales known are in Mexico. Key species are *L. pyriforme* Schaeff. and *L. perlatum* Pers.
Macrolepiota	**13** Food 7 Edible 6 Medicinal 1	Edible species reported from 33 countries; as food in nine (underreported). *Macrolepiota procera* (Scop.) Singer is the key species and most recorded, from around 15 countries on all major

		continents. Locally consumed; trade is essentially small-scale and local
Morchella	**18** Food 14 Edible 4 Medicinal 5	Edible species reported from 28 countries; as food in 10 (underreported). Highly valued genus with several species that fruit in abundance in certain years and are a major source of (export) revenue in several countries. Species are not always eaten in countries where they are collected. Key species *M. esculenta* Fr.
Pleurotus	**40** Food 22 Edible 18 Medicinal 7	Edible species reported from 35 countries; as food in 19 (underreported). Key species is *P. ostreatus* (Jacq. ex Fr.) P. Kumm. in terms of amounts eaten, predominantly from cultivation. Other species said to be tastier. Species occur widely and are regularly picked

		though seldom traded from the wild
Polyporus	**30** Food 15 Edible 9 Medicinal 12	Edible and medicinal species reported from 20 countries; as food or medicine in seven. Many species are regularly used and eaten but of relatively minor importance. Some are cultivated. Only one record known, from Nepal, of selling in markets. No international trade is known to occur
Ramaria	**44** Food 33 Edible 11 Medicinal 5	Edible species reported from 18 countries; used as food in seven. Many records of local use. Regularly sold in markets in Nepal and Mexico and elsewhere. Several major species but perhaps *R. botrytis* (Pers.) Ricken is the most commonly collected and used. Some species are poisonous; others are reported to have

		medicinal properties
Russula	**128** Food 71 Edible 54 Medicinal 25	Edible species reported from 28 countries; as food in 12 (underreported). One of the most widespread and commonly eaten genera containing many edible species. Also poisonous varieties though most can be eaten after cooking. Regularly sold in markets but species names not always recorded. Genus is of tropical origin. Notable species include *R. delica* Fr. and *R. virescens* (Schaeff.) Fr.
Suillus	**27** Food 26 Edible 1 Medicinal 2	Edible species reported from 25 countries; as food in 10 (underreported). Key species is *S. luteus*, exported from Chile. *Suillus granulatus* (L.) Roussel is more widely recorded though its use as a food is limited. Many other species are regularly

		collected and eaten and several are sold in Mexican markets
Terfezia	**7** Food 5 Edible 2	Edible species reported from eight countries; as food in four. Desert truffles occur widely in North Africa and parts of Asia. They are said to be important but few details were found concerning trade or market sales
Termitomyces	**27** Food 23 Edible 4 Medicinal 3	Edible species reported from 35 countries; as food in 16 (underreported). Highly esteemed genus. Many species are widely eaten with often high nutritional value. Collected notably throughout Africa. Used widely in Asia but less well documented. Notable species include *T. clypeatus* R. Heim, *T. microporus* R. Heim and *T. striatus* (Beeli) R. Heim. Sold in

		markets and along roadsides, and good source of income
Tricholoma	**52** Food 39 Edible 13 Medicinal 17	Edible species reported from 30 countries; as food in 11 (underreported). The most important species is *T. matsutake* (Ito et Imai) Sing., in terms of volume collected and financial value. China, both Koreas and the Russian Federation are major exporters to Japan. The Pacific northwest of North America, Morocco and Mexico export related species, but only in significant quantities from the first. Some species are poisonous if eaten raw; others remain so even after cooking. Ignored or poorly regarded in several countries prior to export opportunities, e.g. Bhutan, Mexico (Oaxaca)
Tuber (truffles)	**18**	Edible species

	Food 8 Edible 10	reported from eight countries; as food in four (underreported). Contains species of extremely high value and much esteemed in gourmet cooking, but only of very minor significance to poor communities in the south. There is some interest from Turkey in management of truffles. Scientific principles have been applied to truffle management and successful schemes initiated in Italy, France, Spain, and New Zealand. The “false truffles” comprise other genera, e.g. *Tirmania, Rhizopogon, Terfezia*
Volvariella	**12** Food 5 Edible 7 Medicinal 1	Edible species reported from 27 countries; as food in 7 (underreported, though note possible confusion

between wild and cultivated origins). Key species is *V. volvacea* (Bul. ex Fr.) Singer. Widely cultivated and sold in local markets but also collected from the wild

Information obtained mostly from developing countries. “Food” signifies confirmed use of species; “edible” is a noted property without confirmed consumption. The total number of edible species is the sum of the two. Use refers to country of origin and not countries of export. “Medicinal” confirms use of species for medicinal reasons. Edible species may have medicinal properties and therefore the total number of species in bold may be less than the sum of individual uses. See Lincoff (2002) for distribution of major groups of edible fungi around the world (FAO 2004).

2.4.2 Medicinal Mushrooms

Useful macrofungi comprise species with edible and medicinal properties but distinction between the two categories is not easy. Many of the common edible species have therapeutic properties, thus several medicinal mushrooms are also eaten (see Table 2.3). The total number of useful fungi, defined as having edible and medicinal value, is estimated to be over 2300 species (see Table 2.2) (FAO 2004, 2009).

Ganoderma species are the most valuable medicinal mushrooms, with global values of the produced dietary supplements estimated as US$1.6 billion (Chang & Buswell 1999). *Lentinula edodes* (Berk.) Pegler and *Volvariella volvacea* (Bul. ex Fr.) Singer are also widely cultivated edible fungi with medicinal properties while *Inonotus obliquus* (Ach. ex Pers.) Pilát is the only noncultivated species out of the 25 more used medicinal species (Table 2.4).

Table 2.4 Properties and features of 25 major medicinal macrofungi (FAO 2004).

Species	Medicinal properties	Used as food	Wild collection	Cultivated	Commercial product
Agaricus blazei Murr.	1 Antibiotic	"Edible"	+	Yes	No
Agrocybe aegerita (V. Brig.) Singer	4 Antiviral	Yes	+	Yes	Yes
Armillaria mellea (Vahl) P. Kumm.	4 Antiviral	Yes	++	Yes	Yes
Auricularia auricula-judae (Bull.) J. Schröt.	5 Blood pressure	Yes	++	Yes	Yes
Dendropolyporus umbellatus **(Pers.) Jülich**	4 Antiviral	No	+	Yes	No
Flammulina velutipes (Curtis) Singer	Blood pressure	Yes	++	Yes	Yes
Fomes fomentarius (L.) Fr.	2 Antiinflammatory	No	+	Yes	Yes
Ganoderma applanatum (Pers.) Pat.	4 Antiviral	No	+	Yes	Yes
Ganoderma lucidum (Curtis) P. Karst	11 Hepatoprotective	"Edible"	+	Yes	No

Grifola frondosa (Dicks.) Gray	7 Hypercholesterolemia	Yes	+	Yes	Yes
Hericium erinaceus (Bull.) Persoon	4 Antiviral	Yes	+	Yes	Yes
Hypsizygus marmoreus (Peck) Bigelow	1 Antibiotic	Yes	+	Yes	No
Inonotus obliquus (Ach. ex Pers.) Pilát	4 Antiviral	No	++	No	No
Laetiporus sulphurous (Bull.) Murrill	2 Antiinflammatory	Yes	++	Yes	Yes
Lentinula edodes (Berk.) Pegler	11 Hepatoprotective	Yes	+	Yes	No
Lenzites betulina (L.) Fr.	2 Antiinflammatory	No	?	?No	Yes
Marasmius androsaceus (L. ex Fr.)	2 Antiinflammatory	?Yes	?	?Yes	No
Oudemansiella mucida (Schrad.) Hohn.	1 Antibiotic	"Edible"	++	Yes	No
Piptoporus betulinus	2 Antiinflammatory	No	++	Yes	Yes

(Bull. ex Fr.) P. Karst.					
Pleurotus ostreatus (Jacq. ex Fr.) P. Kumm.	5 Blood pressure	Yes	+	Yes	Yes
Pleurotus pulmonarius (Fr.) Quél.	3 Antitumor	Yes	+	Yes	Yes
Schizophyllum commune Fries	5 Blood pressure	Yes	++	Yes	No
Trametes versicolor (L.) Lloyd	5 Blood pressure	"Edible"	+	Yes	No
Tremella fuciformis Berk.	5 Blood pressure	"Edible"	+	Yes	No
Volvariella volvacea (Bull. ex Fr.) Singer	4 Antiviral	Yes	+	Yes	Yes

+ minor importance; ++ significant amounts collected. Both assessments are in relation to the total amounts used globally, including cultivated production.

Note: The 14 possible medicinal properties consist of: 1 Antibiotic (includes antifungal, antibacterial, antiparasitic but not antiviral); 2 Antiinflammatory; 3 Antitumor; 4 Antiviral; 5 Blood pressure regulation; 6 Cardiovascular disorders; 7 Hypercholesterolemia, hyperlipidemia (high cholesterol, high fats); 8 Antidiabetic; 9 Immune modulating; 10 Kidney tonic; 11 Hepatoprotective; 12 Nerve tonic (antidepressant; vague); 13 Sexual potentiator; 14 Chronic bronchitis (against).

The list of symbiotic macrofungi with medicinal properties is very short in comparison to the number of saprobics, though there is some indication that they have been studied less because they cannot be cultivated (Reshetnikov *et al.* 2001). Out of the 182 medicinal fungi reported by the FAO (2004), only 5% are ectomycorrhizal. This is probably an underestimate for the ectomycorrhizal species (Mao 2000) since research efforts have concentrated on saprobic species that can be cultivated, thus providing a guaranteed supply and uniformity of product.

Mushrooms used in traditional medicine are known as medicinal mushrooms (Ejelonu *et al.* 2013). They produce medically significant metabolites or, nowadays, can be induced to produce such metabolites using biotechnology. Medicinal mushrooms are attracting greater scientific and commercial interest, prompted by a renewed awareness of the use of such material in traditional Chinese medicine (FAO 2004). Edible mushrooms are consumed by humans for their nutritional value and they are also consumed for their presumed medicinal value or, more recently, as functional food for their nutraceutical properties. The medicinal properties of mushrooms depend on several bioactive compounds and their bioactivity depends on how the mushrooms are prepared and eaten. Mushrooms represent a vast source of yet undiscovered potent pharmaceutical products (FAO 2009). The *International Journal of Medicinal Mushrooms* began publication in 1999 and is an important source of information for this expanding field of research (Wasser & Weis 1999a,b).

There has been a spectacular increase of interest and commercial activity concerned with dietary supplements, functional foods, and other products that are "more than just food" (Etkin & Johns 1998; Wasser *et al.* 2000). Mushrooms are increasingly appreciated for their nutritional (Kalac 2009, 2012, 2013) and nutraceutical properties. In addition to culinary and nutritional value, being a food with little fat and very healthy, many research studies demonstrate the benefits that some varieties provide in the human body, strengthening the immune system and fighting diseases like cancer, HIV virus, etc. (includes *A. blazei*, *L. edodes*, *G. lucidum*). Moreover, they are also used for the production of antibiotics and biocontrol of viruses (Anguix 2011).

Beyond nutritional characteristics, mushrooms have also been extensively studied for their medicinal properties, mainly due to their richness in bioactive compounds that present antioxidant, anticancer. and antimicrobial properties, among other bioactivities (Alves *et al.* 2012; Fernandes *et al.* 2015; Ferreira *et al.* 2009. 2010; Heleno *et al.* 2015); for more information see Chapter 4. Although these new products have clear economic potential, their relevance to developing countries is at present still marginal but potentially increasing. Thus per capita value resulting from this production is expected to increase in the medium term (Anguix 2011).

Also noteworthy is the use of certain fungi for bioremediation: soil decontamination, affected by oil spills, as well as biological control of nematodes and insects (Anguix 2011).

Ceremonial and religious roles played by wild fungi in different cultures are closely associated with hallucinogenic properties. Hallucinogenic mushrooms (e.g. psilocybin mushrooms) are occasionally consumed for recreational or religious purposes, but can produce severe nausea and disorientation, and are therefore not commonly considered edible although they are not poisonous. This has attracted much scientific interest (FAO 2004) and analytical work has been carried out to characterize the chemical compounds responsible for the hallucinogenic effects. Alternative uses for those compounds have also been under study.

2.5 Cultivation of Edible Fungi

Edible mushrooms include many fungal species that are either harvested from the wild or cultivated. Easily cultivatable and common wild mushrooms are often available in markets.

Mushroom cultivation started in a very rudimentary manner in Asia about 1000 years ago, where the shiitake was collected in times of mild climate. Subsequently, many attempts were made to grow mushrooms, with uncertain results due to the almost total ignorance of the necessary requirements. In Europe, mushroom cultivation emerged ca. 300 years ago in caves in the area of Paris and later spread to other countries such as Germany, Hungary, etc.

(Anguix 2011). It was not until the second half of the twentieth century that a fundamental transformation occurred at all levels to develop the cultivation of mushrooms.

- Selective substrates for growing mushrooms were developed from agricultural and forestry residues, giving rise to regular production.
- A method of growing mycelium was created with selection of the best suited for cultivation.
- Modern facilities arose where environmental conditions in each of the phases of fungal development were controlled.
- The grower learned and professionalized relevant cultivation techniques.
- A specialized market of fungi with different presentations increased consumption.
- A diversification of fungi and the cultivation of “exotic” fungi occurred.
- As in other sectors of agriculture, mechanization allowed for more consistent production and decreased the need for labor (Anguix 2011).

Within saprobic fungi, two main subgroups exist.

- Primary degraders are those with the ability to initiate the degradation of organic matter.
- Secondary degraders are those that can only synthesize simple substances, generally degraded by the primary degraders. These two subgroups contain the cultivated mushrooms that include some 40 edible species suitable for human consumption, 20 of them exploited industrially but only about six or seven with real commercial importance (Anguix 2011).

There are almost 100 species of saprobic fungi that can be cultivated (Table 2.5). *Agaricus bisporus* (J.E.Lange) Emil J. Imbach, *L. edodes*, and *Pleurotus* spp. dominate commercial markets and account for almost three-quarters of the cultivated mushrooms grown around the world (Chang 1999). The culture of mushrooms offers economic opportunities as well as nutritional and health benefits (Mshigeni & Chang 2000). The main species

cultured are grown on a variety of organic substrates, including waste from the production of cotton and coffee. The technologies are well established and successful mushroom industries have been established in many countries. There has been a huge increase in production over the past decade, especially following increased capacity in China. The cultivation of straw mushrooms (*V. volvacea*) is integrated with rice production in Vietnam. Wherever saprobic species are cultivated, they require a steady supply of raw materials (Pauli 1998).

Table 2.5 Edible and medicinal fungi that can be cultivated (FAO 2004).

Agaricus arvenses Schaeff.	*Hericium coralloides* Scop.	*Paneolus subalteatus* (Berk. & Broome) Sacc.
Agaricus augustus Fr.	*Hericium erinaceum* (Bull.) Persoon	*Paneolus tropicalis* Ola'h
Agaricus bisporus (J.E. Lange) Emil J. Imbach	*Hypholoma capnoides* (Bull.) Persoon	*Phallus impudicus* L.
Agaricus bitorquis (Quélet) Sacc.	*Hypholoma sublateritium* (Schaeff.) P. Kumm.	*Phellinus* spp.
Agaricus blazei Murrill	*Hypsizygus marmoreus* (Peck) Bigelow	*Pholiota nameko* (T. Itô) S. Ito & S. Imai
Agaricus brunnescens Peck	*Hypsizygus tessulatus* (Bull. ex Fr.) Singer	*Piptoporus betulinus* (Bull. ex Fr.) P. Karst.
Agaricus campestris L.	*Inonotus obliquus* (Ach. ex Pers.) Pilát	*Piptoporus indigenus*
Agaricus subrufescens Peck.	*Kuehneromyces mutabilis* (Schaeff.) Singer & A.H. Sm.	*Pleurocybella porrigens* (Pers.) Singer

Agrocybe aegerita (V. Brig.) Singer	*Laetiporus sulphureus* (Bull.) Murrill	*Pleurotus citrinopileatus* Singer
Agrocybe cylindracea (DC.) Maire	*Laricifomes officinalis* (Vill.) Kotl. & Pouzar	*Pleurotus cornucopiae* (Paulet) Rolland
Agrocybe molesta (Lasch) Singer	*Lentinula edodes* (Berk.) Pegler	*Pleurotus cystidiosus* Luis
Agrocybe praecox (Pers.) Fayod	*Lentinus strigosus* Fr.	*Pleurotus djamour* (Rumph. ex Fr.) Boedijn
Albatrellus spp.	*Lentinus tigrinus* (Bull.) Kühner	*Pleurotus eryngii* (DC.) Quél.
Armillaria mellea	*Lentinus tuber-regium* (Rumph. ex Fr.) Singer	*Pleurotus euosmus* (Berk.) Sacc
Auricularia auricula-judae (Bull.) J. Schröt.	*Lepista nuda* (Bull.) Cooke	*Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm
Auricularia fuscosuccinea (Mont.) Henn.	*Lepista sordida* (Schumach.) Singer	*Pleurotus pulmonarius* (Fr.) Quél.
Auricularia polytricha (Mont.) Sacc.	*Lyophyllum fumosum* (Pers. Fr.) PD Orton	*Pleurotus rhodophyllus* Bres
Calvatia gigantea (Batsch ex Pers.) Lloyd	*Lyophyllum ulmarium* (Bull.) Kühner (= *Hypsizygus ulmarium* (Bull.) Redhead)	*Pluteus cervinus* (Schäffer: Fr) P. Kumm.
Coprinus comatus (O.F. Müll.) Pers.	*Macrocybe gigantea* (Massee) Pegler & Lodge (= *Tricholoma giganteum* Massee)	*Polyporus indigenus*
Daedalea quercina	*Macrolepiota*	*Polyporus*

(L.) Pers.	*procera* (Scop.) Singer	*saporema*
Dictyophora duplicata (Bosc) E. Fisch.	*Marasmius oreades* (Bolton) Fr.	*Polyporus umbellatus* (Pers.) Fr.
Flammulina velutipes (Curtis) Singer	*Morchella angusticeps* Peck	*Dendropolyporus umbellatus* (Pers.) Jülich
Fomes fomentarius (L.) Fr.	*Morchella esculenta* Fr.	*Psilocybe cyanescens* Wakefield
Ganoderma applanatum (Pers.) Pat.	*Neolentinus lepideus* (Fr.) Redhead & Ginns (= *Lentinus lepidus*)	*Schizophyllum commune* Fries
Ganoderma curtisii (Berk.) Murrill	*Oligoporus* spp.	*Stropharia rugusoannulatav* Wakefield
Ganoderma lucidum (Curtis) P. Karst	–	*Trametes cinnabarinum* (Jacq.) Fr.
Ganoderma oregonense Murr.	*Oudemansiella radicata* (Relh. ex Fr.) Sing.	*Trametes versicolor* (L.) Lloyd
Ganoderma sinense J.D. Zhao, L.W. Hsu & X.Q. Zhang	*Oxyporus nobilissimus* W.B.Cooke	*Tremella fuciformis* Berk.
Ganoderma tenue J.D. Zhao, L.W. Hsu & X.Q. Zhang	*Panellus serotinus* (Pers.) Kühner (= *Hohenbuehelia serotina* (Pers.) Singer)	*Volvariella bombacyina*
Ganoderma tsugae Murrill	–	*Volvariella volvacea*
Grifola frondosa (Dicks.) Gray	–	*Volvariella volvacea gloiocephala* (Fr.)

= denotes the name as originally published and which has since been changed.

The number of saprophytic cultivated species is steadily increasing, and advice and practical information are readily available (Stamets 2000). The annual global trade in cultivated, saprobic species in 1999 was estimated at US$18 billion (FAO 2004). The economic importance of edible fungi saprobes is not negligible. Species such as button mushroom (*A. bisporus*), oyster mushroom (*P. ostreatus*), king oyster mushroom (*Pleurotus eryngii* (DC.) Quél.) and shiitake (*L. edodes*) are appreciated for their gastronomic quality, and are among the most consumed and marketed. The industrial cultivation of edible fungi saprobes has been achieved with numerous species after the necessary control of environmental conditions such as temperature, humidity, ventilation, and photoperiod, with different needs depending on the species (Fernández-Toirán *et al.* 2011a).

Ectomycorrhizal fungi can also be "cultivated." Trees are inoculated with truffle fungus that then infect the roots and form the ectomycorrhiza. The trees are carefully tended to encourage production of truffles. Methods of "culturing" truffles are constantly being improved (Hall *et al.* 1998).

Table 2.5 lists the 92 names prepared from Stamets (2000) and Chang and Mao (1995). This list contains only saprobic species and excludes ectomycorrhizal species such as truffles (*Tuber* spp.) that are managed in natural habitats.

2.6 Social and Economic Interest in Edible Mushrooms

Wild useful fungi thus contribute to diet, income, and human health. Many species also play a vital ecological role through mycorrhizae, the symbiotic relationships that they form with trees. Truffles and other valuable wild edible fungi (*Chantarellus*,

Lactarius, *Boletus*, *Amanita*, etc.) depend on trees for their growth and cannot be cultivated artificially. The mycorrhizae enable trees to grow in nutrient-poor soils. The importance of wild edible fungi continues to grow for more fundamental reasons. Legal restrictions in several countries have renewed interest in nonwood forest products (NWFP) as an alternative source of income and jobs for people previously employed in forestry. Wild edible fungi have played an important role in providing new sources of income in China, the United States of America, and many other countries (Arora 2008; FAO 2004).

Although the importance of NWFPs is recognized and accepted in Europe, forest research remains mainly focused on timber production. Consequently knowledge about European NWFPs is comparatively scarce, as is research on the ecology, management and economics required to optimize sustainable simultaneous production of different products from forests. A multidisciplinary European network on NWFPs was created in 2014 to help bridge these gaps (COST Action FP1203 2014).

2.7 Edible Mushroom World Production and Commercialization

World production and commercialization of mushrooms are quite difficult to quantify. Most of the production is not officially registered and commercialization occurs in local markets without quantification.

According to FAO statistics, global mushroom production was estimated at about 3.1–7.9 million tons from 1997 to 2012 (Table 2.6), with increasing production during this 15-year period (Figure 2.3), mainly due to increases in Asia and Europe from 2007 to 2012. In the FAO database, mushrooms have been classified as FAOStat code 0449 and have been defined as including, *inter alia*, *B. edulis*, *A. campestris*, *Morchella* spp., and *Tuber magnatum*. Current production can be estimated to be around 7.0 million tons. Asia leads production, followed by Europe and

America (see Table 2.6, Figure 2.3, Figure 2.4). Mushroom production by country shows that China, Italy, USA, The Netherlands, Poland, Spain, France, Ireland, Canada, and UK are the leading producers (Figure 2.5) (FAOStat 2015). The major mushroom-producing countries according to FAO 2012 data are China, Italy, USA, and The Netherlands, accounting for more than 80% of the world production; however, China's share alone is 64% which is more than half of the world mushroom production (see Figure 2.5, Table 2.7).

Table 2.6 Mushroom and truffle production per continent (tonnes) (data from FAOStat 2015).

	1997	2002	2007	2012
Africa	10 846	10 494	14 680	19 440
Americas	434 830	452 155	432 890	470 450
Asia	1 618 006	3 083 575	4 347 798	5 500 705
Europe	981 622	1 132 332	1 142 005	1 913 007
Oceania	42 985	51 912	51 239	56 377
World	**3 088 289**	**4 730 468**	**5 988 612**	**7 959 979**

All figures are aggregates and may include official, semiofficial or estimated data.

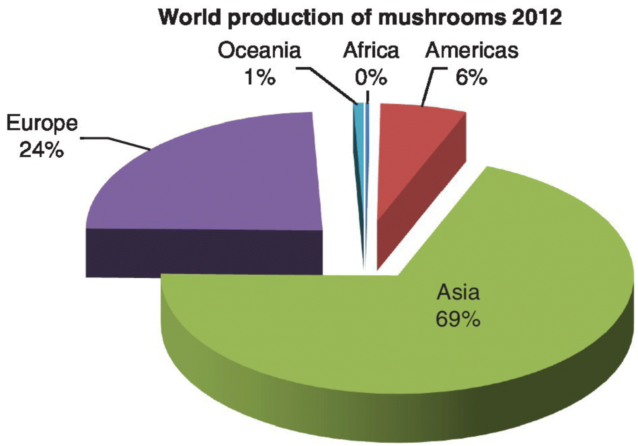

Figure 2.3 Mushroom and truffle relative production per continent (%).

Source: data from FAOStat (2015).

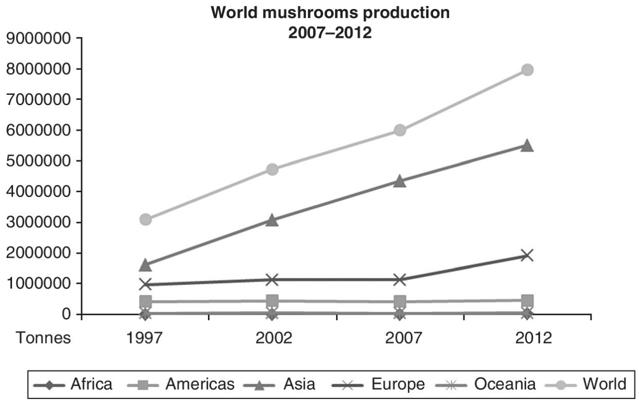

Figure 2.4 Mushroom and truffle production evolution per continent from 1997 until 2012.

Source: data from FAOStat (2015).

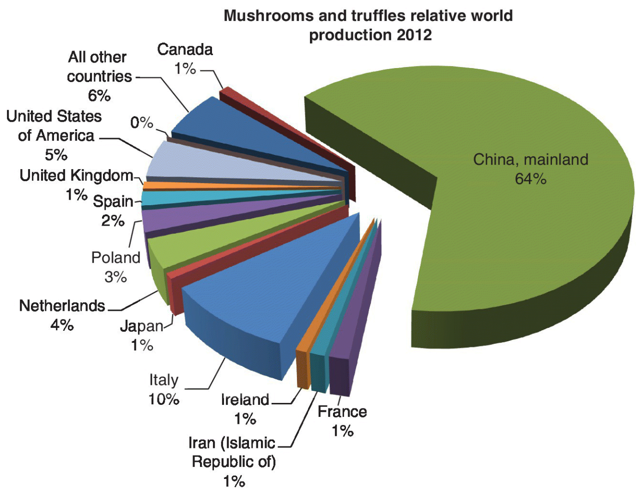

Figure 2.5 Mushroom and truffle relative production (%) per country in 2012.

Source: data from FAOStat (2015).

Table 2.7 Mushroom and truffle production per country (tonnes) (data from FAOStat 2015).

	1997		2002		2007		2012	
Albania	94	Im	104	Im	99	Im	100	F

Algeria	115	Im	123	Im	197	Im	225	F
Armenia	–	M	–	M	–	M	100	F
Australia	35 485	–	43 412	–	42 739	–	46 493	–
Austria	300	–	500	–	900	–	1600	–
Azerbaijan	–	M	–	M	1500	F	1500	F
Belarus	–	M	5000	F	6800	F	7000	F
Belgium	32 938	**	42 500	–	43 361	Im	42 000	F
Bosnia and Herzegovina	700	F	1000	F	1000	F	994	F
Brunei Darussalam	5	Im	9	F	9	F	11	F
Bulgaria	13 000	F	8961	Im	1716	–	2093	–
Canada	68 020	–	75 075	–	73 260	–	82 000	F
China, Hong Kong SAR	37	Im	44	Im	30	F	37	F
China, Mainland	1 450 000	F	2 850 000	F	4 060 000	F	5 150 000	F
China, Taiwan Province of	12 159	–	9762	–	8488	–	8773	–
Croatia	–	M	–	M	–	M	M	
Cyprus	2170	–	1270	–	1213	–	701	–
Czech Republic	300	F	800	–	313	Im	361	F
Democratic People's Republic of Korea	4310	Im	6200	F	6500	F	5700	F
Denmark	8766	–	8686	–	11 000	F	10 700	F
Estonia	60	F	100	*	100	F	130	F
Finland	1242	–	1756	–	2016	–	1536	–
France	173	–	175	–	162	–	116	–

	000		288		450		574	
Germany	69 000	–	62 000	–	55 000	–	52 907	–
Greece	485	Im	500	–	3500	F	3400	F
Hungary	13 559	–	20 257	–	21 637	–	19 330	–
Iceland	293	–	450	–	575	–	646	–
India	9000	F	40 000	–	37 000	–	41 000	F
Indonesia	19 000	F	18 300	–	48 247	–	40 659	–
Iran (Islamic Republic of)	8962	Im	21 000	F	28 000	–	87 675	–
Ireland	57 800	–	69 000	–	81 000	F	67 063	F
Israel	1260	–	7800	–	9500	–	10 000	–
Italy	57 646	–	72 700	–	85 911	–	78 500	F
Japan	74 782	–	64 400	–	67 000	F	61 500	F
Jordan	500	–	700	–	852	Im	1150	F
Kazakhstan	–	M	500	*	524	Im	540	*
Kyrgyzstan	930	Im	1662	Im	225	Im	200	F
Latvia	367	Im	500	*	500	F	500	F
Lithuania	2398	Im	2900	*	6688	–	4200	–
Luxembourg	–	–	15	–	5	–	5	–
Madagascar	1100	F	1054	Im	1651	Im	2087	F
Malta	–	M	644	Im	1093	–	1342	–
Mongolia	–	M	30	*	217	Im	278	F
Montenegro	–	–	–	–	600	F	600	F
Morocco	1662	Im	1850	F	1878	Im	2045	F
Netherlands	240 000	–	270 000	–	240 000	–	307 000	–
New Zealand	7500	F	8500	–	8500	F	9884	F
Philippines	762	–	560	F	565	F	509	–
Poland	101 786	Im	120 000	F	180 000	F	220 000	F
Portugal	1160	Im	1143	Im	1050	F	1240	F
Republic of Korea	13 181	–	24 688	–	28 764	–	26 000	–

Republic of Moldova	193	Im	349	Im	400	F	500	F
Réunion	70	Im	38	Im	43	Im	61	F
Romania	4000	F	9000	F	1083	–	9311	–
Russian Federation	1500	*	7000	*	5700	*	5000	–
Serbia	8000	F **	15 605	Im**	4500	F	5000	F
Singapore	–	M	M	17	–	57	–	
Slovakia	700	F	800	F	1500	F	1898	F
Slovenia	1833	Im	1505	Im	894	Im	1100	F
South Africa	7406	–	7021	–	10 320	–	14 284	–
Spain	81 304	–	134 669	–	131 974	–	146 000	–
Switzerland	7239	–	7400	*	7440	–	7977	–
Thailand	9000	F	9035	Im	6394	Im	6820	F
Republic of Macedonia	1600	F	3000	F	2500	F	2800	F
Tunisia	67	Im	88	Im	107	Im	125	F
Turkey	1200	F	11 000	F	23 426	–	33 825	–
Ukraine	2000	*	3500	*	7200	*	14 000	*
United Kingdom	107 359	–	84 700	–	71 500	–	73 100	–
United States of America	366 810	–	377 080	–	359 630	–	388 450	*
Uzbekistan	400	F	316	Im	509	Im	670	F
Vietnam	10 339	Im	16 299	Im	18 818	Im	23 000	F
Zimbabwe	426	Im	320	F	484	Im	613	F
TOTAL	**475 436**	**1 693 978**	**1 748 513**	**2 617 053**				

* unofficial figure; F, FAO estimate; Im, FAO data based on imputation methodology; M, data not available.

** Serbia and Montenegro.

*** Belgium-Luxembourg.

In the USA and Europe, the major contribution to mushroom production is made by the white button mushroom, *A. bisporus*. In Asian countries the scenario is different and other species are also cultivated for commercial production (Wakchaure 2011).

Data from the Chinese Association of Edible Fungi possibly include all these mushrooms. Consequently the mushroom production figures quoted by Chinese are at a much higher scale. This does emphasize the contribution of other edible mushrooms/medicinal mushrooms, even if the figures may seem exaggerated. Mushroom export from China accounts for less than 5% of its total domestic production and about half of it is to Asian countries; 95% of mushroom production in China is for local consumption, with a potential per capita value of over 10 kg/person/year. This is much higher than most of the European countries and the USA where it is around 3 kg/person/year (Wakchaure 2011).

World mushroom production (FAOStat 2015) is continously increasing, from 0.30 to 7.2 million tons over the last 50 years, from 1961 to 2012 (Figure 2.6), in line with exports/imports that showed a marginal increase up to 1985 and a tremendous increase beyond that up to 2012. Poland, The Netherlands, Ireland, China, Belgium, Lithuania, Canada, and USA are the major mushroom-exporting countries while countries including UK, Germany, France, The Netherlands, Belgium, Russian Federation, and Japan are the major importers. Processed mushroom (canned and dried) export is continuously increasing, from 0.049 to 0.683 million tons over the period of the last four decades (1970 – 2010), compared to fresh mushroom exports (0.014 to 0.482 million tons), but fluctuations in export are higher for processed mushrooms. In the USA, five decades ago, 75% of mushroom consumption was in the form of canned product. Today, canned mushroom contributes only 15% of total mushroom consumption. The consumption of canned mushroom is static and that of fresh mushroom has increased continuously. This clearly shows that consumers are interested in shifting towards fresh mushroom consumption (Wakchaure 2011).

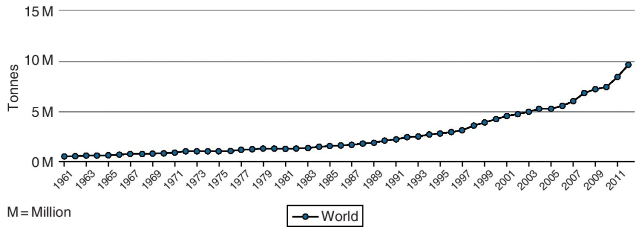

Figure 2.6 Mushroom and truffle world production from 1961 to 2012.

Source: data from FAOStat (2015).

The European Union mushroom production was about 24% of world production in 2012 (FAOStat 2015). Italy is the largest producer, Poland is the largest exporter, UK the largest importer; France and Spain are also among the larger European producers as well as consumers. Per capita consumption in these countries is very high (about 3.5 kg) (Wakchaure 2011). Highest per capita consumption of mushroom is in The Netherlands (11.62 kg) followed by Ireland (6.10 kg) and Belgium (4.46 kg). As a comparison of different patterns of consumption, the per capita consumption of mushroom in India increased from 25 g to 40 g in the 10 years 1996– 2007. However, Indian estimates of per capita consumption are about 90 g, which is still much less compared to other countries including the USA (1.49 kg) and China (1.16 kg) (Wakchaure 2011).

A case study from a region of Spain where management of mycological resources has been regulated since 2003, Castilla and Leon, showed the number of species with potential economical interest beyond the species quantified by FAOStat data and the importance of the direct and indirect profits coming from mushrooms (Table 2.8). The production of Castilla and Léon Province is up to 31.466 tons per year, with an associated direct income of 80 160 M€ and an indirect profit from micotourism around 4 650 724 € (see Table 2.8) (Martinez-Peña *et al.* 2011).

Table 2.8 Wild edible mushroom production (except truffles), commercialization and economical value (including micotourism) in the province of Castille and Leon, Spain (Martinez-Peña 2011).

Edible	Production	Economical	Micotourism	FTE

species	(tonnes)	value € (×1000)	income (€)	micotourism
Agaricus spp.	735	4.735	–	–
Amanita caesarea (Scop.) Pers.	1972	8.322	–	–
Boletus aereus Bull.	1879	7.683	–	–
Boletus reticulatus Schaeff.	328	1.263	–	–
Boletus edulis Bull.	1564	6.021	–	–
Boletus pinophilus Pilát & Dermek	1035	6.292	–	–
Calocybe gambosa (Fr.) Donk	23	187	–	–
Cantharellus cibarius Fr.	324	2.268	–	–
Helvella spp.	26	26	–	–
Hygrophorus marzuolus (Fr.) Bres.	589	4.596	–	–
Hygrophorus spp.	9830	9.830	–	–
Lactarius deliciosus (L. ex Fr.) S.F. Gray	5522	16.622	–	–
Lepista spp.	1248	1.248	–	–
Macrolepiota	591	591	–	–

spp.				
Marasmius oreades (Bolton) Fr.	86	432	–	–
Morchella spp.	613	6.131	–	–
Pleurotus eryngii (DC.) Quél	317	1.558	–	–
Tricholoma portentosum (Fr.) Quél.	786	2.358	–	–
TOTAL	**31.466**	**80.160**	**4.650.724**	**180**

FTE, full-time equivalent.

It is clear from the above that the EU and USA are the biggest markets and Poland and China are the biggest competitors in the mushroom market (Wakchaure 2011). These production and commercialization values are underestimated if we consider all the remaining species not quantified by FAOStat but collected and commercialized around the world as shown by the Spanish data.

2.8 Conclusion

Fungi are an ancient group of organisms and their earliest fossils are from the Ordovician, 460–455 million years old (Redecker *et al.* 2000). Based on fossil evidence, the earliest vascular land plants appeared approximately 425 million years ago, and it is believed that fungi may have played an essential role in the colonization of land by these early plants.

Estimates for the number of fungi species in the world ranges from 3.5 to 5.1 million species and the Dictionary of the Fungi (Kirk *et al.* 2008) reported 98 998 species of all described fungi.

Since time immemorial, a considerable number of identified

species of fungi have made a significant contribution to human food and medicine.

The major features of wild edible fungi based on the first global assessment by the FAO (2004) are:

- 2327 wild useful species recorded; 2166 are edible and 1069 used as food, with at least 100 other "known food" species still lacking published studies
- 470 species have medicinal properties, of which 133 are neither eaten or said to be edible; a further 181 species have other properties and uses valued by people, e.g. religious, as tinder
- they are collected, consumed, and sold in over 80 countries worldwide
- amounts collected each year globally are several million tons with a minimum value of US$2 billion, which has consistently increased since the first FAO records in 1961 (FAOStat 2015).

The major benefits of wild edible fungi are:

- valuable sources of nutrition, often with associated health benefits
- important source of income for local communities and national economies
- key species being ectomycorrhizal and helping to sustain tree growth and healthy forests
- being particularly valuable to rural people in developing countries.

The most important topics that need further investigation in mycology include diet, fungal ecology (mycorrhizas), and storage. How to manage wild edible fungi, to achieve sustainable production for both commercial harvesting and subsistence uses are key issues that need more work to support effective management.

Some factors related to mushroom biodiversity have been discussed, starting with the origin and diversity of fungi, through ecological diversity and diversity of habitats and global diversity of soil fungi, to finally focus on wild edible fungi, their diversity

and social and economic interest. Cultivation aspects were also referred to, both concerning edible and medicinal fungi that can be cultivated and the general features of cultivation of edible fungi. Edible mushroom world production and commercialization were also presented with a statistical approach to FAOStat data from 1997 to 2012.

The main priorities of research on wild edible fungi are currently much the same as they were in the FAO 2004 report:

1. identification of species
2. nutritional status
3. mycorrhizae
4. storage
5. effective management
6. nutraceutical and medicinal applications.

Our research group has been intensively working and publishing on topics 2, 3, 4, and 6.

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3 The Nutritional Benefits of Mushrooms

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3.1 Introduction

Wild edible mushrooms are appreciated and consumed in different parts of the world, not only for their delicate organoleptic qualities, but also for their chemical and nutritional characteristics (Maga 1981; Manzi *et al*. 2001). The culinary value of mushrooms is due mainly to their organoleptic properties such as odor, flavor, and texture (Guedes de Pinho *et al*. 2008; Maga 1981). Regarding nutritional qualities, mushrooms stand out due to their amino acid composition (Chang & Miles 2004; Crisan & Sands 1978; Kalač 2009), which is considered of high biological value and can be correlated to animal proteins (Gruen & Wong 1982). This consideration is relatively important due to increasing disease prevalence all over the world associated with high meat consumption. However, the potential nutritional value and the implication of a gradual substitution of meat with mushrooms requires careful examination involving detailed chemical and biological studies (Aletor 1995). Mushrooms' chemical characteristics associated with pharmacological uses have also been widely studied (Bobek & Galbavy 1999; Bobek *et al*. 1991, 1995). All these special features, in addition to their variable

colors, particular shapes, and rarity of several wild species only present in specific geographical areas, make mushrooms a very valuable resource, with importance for gourmet cooking in many parts of the world, where dishes prepared from wild mushrooms can achieve high prices on the market (Hall *et al*. 2003).

Consumption of wild edible mushrooms goes back to the beginnings of civilization and has been developed in many countries around the world, especially in China, Japan, United States, Spain, and Italy (Boa 2004; Wang 1987). In Latin America, Mexico, and, to a lesser extent, in Guatemala and Honduras, people have a deeply rooted mycological knowledge (Estrada-Martínez *et al*. 2009; Ruan-Soto *et al*. 2004), and the diversity of species present in these countries has been incorporated into several activities such as cooking, traditional medicine, and especially religious rituals (Villarreal & Pérez-Moreno 1989). Mushrooms constitute an important source of food and monetary income in developing as well as developed countries, especially those having important forest resources (Boa 2004; Hosford *et al*. 1997; Wong *et al*. 2001). For small rural communities, selling wild edible mushrooms allows families to work toether, generating complementary incomes in a diversified economic strategy, or being the main income during the rainy season (Martínez-Carrera 2010).

This chapter presents information on the nutritional composition of different wild edible mushroom species taken from reports from different authors, and also the potential benefits they can provide as a source for the human diet.

3.2 Nutritional Properties of Mushrooms

Scientific studies indicate that mushrooms are a healthy food source, having low calorie and fat content. They have a high protein content with an important ratio of essential amino acids, dietary fibers, carbohydrates, vitamins, and minerals (Agrahar-Murugkar & Subbulakshmi 2005; Barros *et al*. 2008; Heleno *et al*. 2009; Kalač 2009; Ouzouni & Riganakos 2007; Reis *et al*. 2012). Investigation of nutritional composition includes

determination of macronutrients such as proteins, amino acids, dietary fibers, lipids, carbohydrates, ash, as well as micronutrients, namely vitamins and minerals, among others, which are determined and analyzed following the methods suggested by the AOAC (2005).

Chemical composition of mushroom species may be affected by several variables such as genetic structure, strains, maturation stage, environmental conditions, such as soil composition, as well as the specific part of the mushroom, postharvest preservation method (dry or fresh procedures), and cooking process (Barros *et al*. 2007b; Chang & Miles 2004; Crisan & Sands 1978; Manzi *et al*. 2001, 2004).

Dry weight (dw) content of fresh mushrooms is relatively low, around 10%, and mainly consists of carbohydrates, proteins, dietary fibers, and minerals (Wang *et al*. 2014). Besides, since fresh fructifications provide about 90% of moisture content, data on chemical composition of mushrooms, usually need to be normalized according to dry matter content (Chudzyński & Falandysz 2008).

3.2.1 Proteins and Amino Acids

The nutritional value of mushrooms is directly related to their protein content. According to a report published by the Food and Agriculture Organization (FAO 1991), mushroom protein has better nutritive quality than that from vegetables. Crisan and Sands (1978) proposed a "nutritional index" to determine food nutritional values based on the amount and quality of amino acid fraction (EAA Index), as a way of solving difficulties related to comparing mushrooms with low amounts of proteins with high nutritional value with those having high amounts of low nutritional value proteins. Most of the edible mushrooms with a high EAA Index could be placed near to meat and milk, while those having a low EEA Index value can be placed between vegetables and legumes (Chang & Miles 2004).

Protein determination could represent a problem since different conversion factors have been used calculated on the base of

nitrogen content. The crude protein content of most foods is currently calculated from the nitrogen content adjusted by a conversion factor (N × 6.25) assuming that proteins contain 16% of digestible nitrogen with insignificant amounts of nonprotein nitrogen. However, the cell walls of fungi contain an important amount of nonprotein nitrogen in the form of chitin. Therefore, another conversion factor applied to mushroom is N × 4.38, based on the presence of 70% of digestible protein (0.7 × 6.25 = 4.38) (Barros *et al*. 2007a, 2008; Breene 1990). Furthermore, Bauer-Petrovska (2001) recommended another factor (N × 4.16), which was proposed by observing a mean proportion of 33.4% of nonprotein nitrogen (from total nitrogen) in numerous samples. In this way, in some articles, crude protein is thus overestimated. Crude protein values for wild mushrooms, depending on the species analyzed, range between 12.0 and 59.4 g/100 g dw, as in the case of *Sarcodon aspratus* (Berk.) S. Ito (Zhang & Chen 2011) and *Lepista nuda* (Bull.) Cooke (Barros *et al*. 2008), respectively.

Examples of crude proteins for four different mushroom species are presented in Table 3.1, displaying variations in this macronutrient value calculated for one species by different authors, depending on the protein conversion factor used. Kalač (2009) warns that when this value is overestimated in some studies, it mainly affects the carbohydrate value if calculated by difference through the following equation: 100% – (% moisture + % crude protein + % lipids + % ash).

Table 3.1 Crude protein content using different conversion factors for four species of wild edible mushroom species (g/100 g dry weight).

Species	Crude protein (N factor used)	Reference
Amanita rubescens Pers.	31.9 (N × 6.25)	Colak *et al*. 2007
	26.0 (N × 4.38)	Ouzouni & Riganakos 2007
	17.4 (N × 4.38)	León-Guzmán *et al*. 1997

Cantharellus cibarius Fr.	53.7 (N × 4.38)	Barros *et al.* 2008
	34.1 (N × 6.25)	Colak *et al.* 2009
Lepista nuda (Bull.) Cooke	19.8 (N × 4.38)	Ouzouni & Riganakos 2007
	44.2 (N × 6.25)	Colak *et al.* 2007
	59.4 (N × 4.38)	Barros *et al.* 2008
Lycoperdon perlatum Pers.	17.2 (N × 4.38)	Barros *et al.* 2008
	44.9 (N × 6.25)	Colak *et al.* 2009

The distribution of proteins within a fruiting body and changes in protein content during the development of the fruiting body remain mostly unclear. Vetter and Rimóczi (1993) reported the highest crude protein content, together with the highest digestibility (92%), in cultivated *Pleurotus ostreatus* (Jacq.) P. Kumm. (oyster mushroom) at a cap diameter of 5–8 cm. At that stage of development, crude protein contents were 36.4 and 11.8 g/100 g dw in cap and stipe, respectively. Thereafter, both crude protein and its digestibility decreased.

Proteins consist of over 20 amino acids in variable amounts. Humans can convert some of these amino acids into others but nine of them are considered as essential amino acids (lysine, methionine, tryptophan, threonine, valine, leucine, isoleucine, histidine, and phenylalanine). Free amino acid content is relatively low in mushrooms, only representing about 1% dry matter, and for this reason, their nutritional contribution for the human diet is limited (Kalač 2009). However, some authors point out that mushrooms are a good source of these compounds (Cheung 2010; Heleno *et al.* 2009), probably because the amount of free amino acids in wild mushrooms is highly affected by environmental factors.

Moreover, the amount and type of amino acids vary according to fungal species. For example, Mdachi *et al.* (2004) indicate that leucine was abundantly found, between 32% and 28% of the total essential amino acid content, in *Boletus pruinatus* Fr. & Hök and *Boletinus cavipes* (Opat.) Kalchbr, and the second most abundant essential amino acid was valine, recorded between 23% and 21%.

Moreover, Ayaz *et al*. (2011) found that among the essential amino acids, leucine was the most abundant (48%) in *Agaricus arvensis* Schaeff.

On the other hand, other studies indicate that proteins in wild edible mushrooms contain considerable amounts of nonessential amino acids, such as in *Amanita rubescens* Pers. (73.16%), *Boletus frostii* J.L. Russell (81.83%), and *Ramaria flava* (Schaeff.) Quél. (81.86%) (León-Guzmán *et al*. 1997). Data on essential and nonessential amino acids for some wild edible mushrooms are given in Tables 3.2 and 3.3, respectively.

Table 3.2 Essential free amino acid content (g/100 g dry weight) in some edible wild mushroom species.

Species	**Val**	**Leu**	**Thr**	**Ile**	**Lys**	**Trp**	**Met**	**Phe**	**References**
Boletinus cavipes (Opat.) Kalchbr.	7.96	10.6	7.79	–	3.43	2.23	2.31	3.32	Mdachi *et al*. 2004
Boletus pruinatus Fr. & Hök	6.04	8.40	5.02	–	2.59	2.94	1.53	nd	Mdachi *et al*. 2004
Clitocybe maxima (P. Gaertn., G. Mey. & Scherb.) P. Kumm.)	3.74	0.44	7.24	Nd	0.79	8.37	nd	6.91	Liu *et al*. 2012
Craterellus cornucopioides (L.) Pers.	0.41	17.5	16.37	0.08	8.09	nd	12.74	nd	Liu *et al*. 2012
Laccaria	7.99	16.83	12.82	Nd	11.97	0.73	2.59	7.31	Liu

amethystea (Bull.) Murrill										*et al.* 2012
Pleurotus sajor-caju (Fr.) Singer	7.81	0.43	8.56	–		6.33	0.41	nd	nd	Mdachi *et al.* 2004

Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; nd, not detected; Phe, phenylalanine; Thr, threonine; Trp, tryptophan; Val, valine.

Table 3.3 Nonessential free amino acid content (g/100 g dry weight) in some wild edible mushroom species.

Species	**Ala**	**Arg**	**Asp**	**Glu**	**Gly**	**Ser**	**Tyr**	**Cys**	**Reference**
Clitocybe maxima (P. Gaertn., G. Mey. & Scherb.) P. Kumm.	18.62	0.08	2.72	0.90	11.69	nd	9.42	2.74	Liu *et al.* 2012
Craterellus cornucopioides (L.) Pers.	0.86	nd	0.54	7.67	5.71	16.79	0.82	0.34	Liu *et al.* 2012
Boletinus cavipes (Opat.) Kalchbr.		1.49	10.0	–	8.37	8.93	4.78	–	Mdachi *et al.* 2004
Boletus pruinatus Fr. &	11.5	0.03	8.36	15.4	6.14	7.42	3.42	–	Mdachi *et al.* 2004

Hök									
Laccaria amethystea (Bull.) Murrill	10.32	nd	0.49	13.12	2.20	10.34	nd	2.57	Liu *et al.* 2012

Ala, alanine; Arg, arginine; Asp, aspartic acid; Cys, cystine; Glu, glutamic acid; Gly, glycine; nd, not detected; Ser, serine; Tyr, tyrosine.

Other amino acids that affect the flavor of mushrooms are glutamic and aspartic acids (Maga 1981; Mau *et al.* 2001). Glutamic acid and alanine were reported as the prevailing free amino acids in *Tricholoma portentosum* (Fr.) Quél. and *T. terreum* (Schaeff.) P. Kumm. (Díez & Alvarez 2001).

3.2.2 Carbohydrates: Available Carbohydrates and Dietary Fiber

Nutritionally, it is important to differentiate between two broad categories of carbohydrates: the "available carbohydrates", which are digested and absorbed by the small intestine and provide energy to the human body cells, and "dietary fiber," considered as nondigested carbohydrates that pass through to the large intestine, forming the substrate for colonic microflora fermentation. Available carbohydrates include monosaccharides, disaccharides, trisaccharides, starch, and some maltooligosaccharides. Dietary fiber includes nonstarch polysaccharides such as cellulose, hemicellulose, pectins, gums, mucilages, β-glucans, oligosaccharides, and chitin (EFSA 2010).

Carbohydrates represent about 50% of mushroom dry weight (Kalač 2013). They play a major role in mushroom cell energetic metabolism, and can also be used for storage and structural polysaccharide synthesis (Lehninger *et al.* 2008). Data on carbohydrates for some wild edible mushrooms are given in Table 3.4. Grangeia *et al.* (2011) have reported that the Mycorrhizal species they studied presented a higher total sugar content (16–42 g/100 g dw) than saprotrophic mushrooms (0.4–15 g/100 g dw). Available carbohydrates include mannitol (0.3–5.5 g/100 g dw as reported by Vaz *et al.* 2011) and trehalose (9.3–42.8 g/100 g dw

as reported by Vaz *et al*. 2011) as the main representatives of polyols and oligosaccharides, respectively (Kalač 2013), and also, glucose (0.5–3.6 g/100 g dw as reported by Kim *et al*. 2009), and glycogen (5–10 g/100 g dw as reported by Kalač 2013). Anyway, different authors present divergent values for the same or different especies, as shown in Table 3.5.

Table 3.4 Recent data on approximate composition (g/100 g dry weight) and energy value (kcal/100 g dry weight) for some wild edible mushroom species.

Species	Proteins	Lipids	Ash	Carbohydrates	Energy value	References
Agaricus campestris L.	18.57	0.11	23.16	58.16	–	Pereira *et al*. 2012
Armillaria mellea (Vahl) P. Kumm.	16.38	5.56	6.78	71.28	400. 68	Vaz *et al*. 2011
Boletus aereus Bull.	17.86	0.44	8.87	72.83	366.69	Heleno *et al*. 2011
Boletus edulis Bull.	21.07	2.45	5.53	70.96	390.11	Heleno *et al*. 2011
Calvatia utriformis (Bull.) Jaap	20.37	1.90	17.81	59.91	338.26	Grangeia *et al*. 2011
Coprinus comatus (O.F. Müll.) Pers.	15.67	1.13	12.85	70.36	354.27	Vaz *et al*. 2011
Flammulina	17.89	1.84	9.42	70.85	–	Pereira

velutipes (Curtis) Singer						*et al.* 2012
Lactarius deliciosus (L.) Gray	20.20	8.02	7.15	64.63	_	Akata *et al.* 2012
Russula olivacea (Schaeff.) Fr.	16.84	1.99	37.78	43.38	258.84	Grangeia *et al.* 2011

Table 3.5 Soluble sugars content (g/100 g dry weight) in different wild edible mushroom species.

Species	**Trehalose**	**Mannitol**	**Arabinose**	**Fructose**	**References**
Agaricus campestris L.	3.62	16.94	_	nd	Pereira *et al.* 2012
Armillaria mellea (Vahl) P. Kumm.	9.33	5.45	0.78	_	Vaz *et al.* 2011
Boletus aereus Bull.	1.34	4.65	nd	_	Heleno *et al.* 2011
Boletus edulis Bull.	2.45	12.40	nd	_	Heleno *et al.* 2011
Calvatia utriformis (Bull.) Jaap	0.40	nd	_	nd	Grangeia *et al.* 2011
Coprinus comatus (O.F. Müll.) Pers.	42.82	0.40	nd	_	Vaz *et al.* 2011

Flammulina velutipes (Curtis) Singer	15.08	5.98	_	nd	Pereira *et al*. 2012
Russula olivacea (Schaeff.) Fr.	0.71	15.25	_	0.23	Grangeia *et al*. 2011

nd, not detected.

Polyols, mainly mannitol, which are responsible for the development and growth of the fruiting bodies (Barros *et al*. 2008), have half of the calories of common soluble sugars; since they are poorly absorbed by the human body, they do not raise insulin levels in blood and do not promote tooth decay (Dikeman *et al*. 2005). Glycogen is the reserve polysaccharide of mushrooms but, as it is widely consumed, mainly in meat, its low intake from mushrooms seems to be nutritionally negligible. Other sugars such as fructose and arabinose have been detected in different species of edible mushrooms, generally in lower amounts than mannitol and trehalose (see Table 3.5).

The total dietary fiber (TDF) is the sum of intrinsic nondigestible carbohydrates, soluble and insoluble fractions. The terms "soluble" and "insoluble" have been used in the literature to classify dietary fiber as viscous soluble in water (e.g. pectins) or as water insoluble (e.g. cellulose). In this way, mushrooms include soluble dietary fibers (SDF), such as oligosaccharides (mainly trehalose), β-glucans and manans, and insoluble dietary fibers (IDF), mainly chitin. The proportion of each dietary fraction varies according to species, but in general terms, IDF shows higher levels than SDF (Manzi *et al*. 2001). The study of Sanmee *et al*. (2003) involving 13 species of wild edible mushrooms reported TDF values between 8.3 g/100 g dw for *Craterellus odoratus* (Schwein.) Fr. and 16.8 g/100 g dw for *Heimiella retispora* (Pat. & C.F. Baker) Boedijn. Nile and Park (2014), analyzing 20 species of wild growing edible mushrooms in India, reported a TDF range between 24 and 37 g/100 g dw corresponding to *Lactarius sanguifluus* (Paulet) Fr. and *Pleurotus djamor* (Rumph. ex Fr.)

Boedijn, respectively, an IDF range of 12–21 g/100 g dw and an SDF range of 2–4 g/100 g dw. The composition of TDF in electron beam-irradiated samples of *Macrolepiota procera* (Scop.) Singer and *Boletus edulis* Bull. ranged between 29.1–33.9 g/100 g dw and 26.7–30.8 g/100 g dw, respectively (Fernandes *et al*. 2015). Wild species of the genus *Boletus* when raw (dehydrated and rehydrated) showed higher levels of IDF (2.28–8.99 g/100 g edible weight) and SDF (0.32–2.20 g/100 g edible weight) compared with other fresh cultivated species; the effect of cooking on their chitin content was not significant (Manzi *et al*. 2004).

The fairly high detected levels of dietary fiber in these mushrooms might be considered as a desirable characteristic, since fiber plays an important role in the human diet (EFSA 2010). Insoluble dietary fiber improves the functioning of the digestive tract, by cleaning waste stuck to the intestine walls and increasing fecal volume. Soluble dietary fiber, besides capturing water, diminishes and slows fat and sugar absorption from food, which helps to regulate cholesterol and glucose levels in the blood (Cho 2001).

Regarding water-insoluble fiber, chitin is a structural N-containing polysaccharide that accounts for up to 80–90 g/100 g dw in mushroom cell walls (Kalač 2013). Trehalose, as part of SDF, is common to most immature fructification, being a reserve sugar that is metabolized as fructifications mature (see Table 3.5).

3.2.3 Lipids

The content of total lipids (crude fat) is low in mushrooms compared with the other macronutrients (see Table 3.4), and ranges from 0.11 to 8.02 g/100 g dw in wild *Agaricus campestris* L. (Pereira *et al*. 2012) and *Lactarius deliciosus* (L.) Gray (Akata *et al*. 2012) as reviewed by Kalač (2013). Lipids play a fundamental role in the human body; they act as hormones or as their precursors, helping the digestion process, and constitute a source of metabolic energy (Burtis *et al*. 2008). In general, crude fat content is represented by all sorts of lipidic compounds, including free fatty acids, monoglycerides, diglycerides, triglycerides, sterols, and phospholipids.

Fatty acids are the basic components of most lipids, and in mushrooms, polyunsaturated linoleic acid (C18:2, ω6), monounsaturated oleic acid (C18:1, ω9) and nutritionally undesirable saturated palmitic acid (C16:0) prevail (Kalač 2009). Many authors report that unsaturated fatty acids predominate over saturated (Barros *et al* 2008; Ribeiro *et al*. 2009 Yilmaz *et al*. 2006). Linoleic (ω6) and α-linoleic (ω3) acids are essential polyunsaturated fatty acids (PUFA) since they cannot be synthesized by humans and must be ingested with food. Both compounds are highly correlated with metabolic functions, lowering the risk of cardiovascular diseases, triglyceride level, hypertension, and arthritis (Voet & Voet 2004; Wang *et al* 2003). Though linoleic acid level is generally low in mushrooms (Yilmaz *et al*. 2006), it greatly contributes to mushroom flavor on account of its role as a precursor of 1-octen-3-ol, which is the main aromatic compound of most mushrooms (Guedes de Pinho *et al*. 2008; Maga 1981).

Table 3.6 shows some of the main saturated and unsaturated fatty acids present in different wild edible mushroom species. The high proportion of unsaturated fatty acids in *Coprinus comatus* (O.F. Müll.) Pers. (74.86%) (Vaz *et al*. 2011), *Calvatia utriformis* (Bull.) Jaap Pers. (70.29%) (Grangeia *et al*. 2011), and *Agaricus campestris* (68.97%) (Pereira *et al*. 2012) is mainly due to the presence of linoleic acid.

Table 3.6 Total fatty acids composition (relative percentage, %) for some wild edible mushroom species.

Species	**Fatty acids**					**References**
	C16:0 SFA	**C16:1** MUFA	**C18:0** SFA	**C18:1** MUFA	**C18:2** PUFA	
Agaricus campestris L.	12.48	–	2.73	6.09	68.97	Pereira *et al*. 2012
Armillaria mellea (Vahl) P. Kumm.	11.04	6.36	3.53	47.74	27.71	Vaz *et al*. 2011

Boletus aereus Bull.	12.47	0.58	3.80	36.72	43.83	Heleno *et al*. 2011
Boletus edulis Bull.	9.57	0.55	3.11	42.05	41.32	Heleno *et al*. 2011
Calvatia utriformis (Bull.) Jaap	13.54	0.22	2.43	6.00	70.29	Grangeia *et al*. 2011
Coprinus comatus (O.F. Müll.) Pers.	10.56	0.59	1.90	6.27	74.86	Vaz *et al*. 2011
Flammulina velutipes (Curtis) Singer	10.31	–	1.38	15.08	56.33	Pereira *et al*. 2012
Russula olivacea (Schaeff.) Fr.	16.13	1.31	2.78	25.99	50.20	Grangeia *et al*. 2011

C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acids.

3.2.4 Energetic Value/Caloric Content

Mushrooms are appreciated because of their low caloric content, usually 350–400 kcal/100 g dw (Kalač 2013). According to what is shown in Table 3.4, the range of energetic contribution varies from 258.84 to 400.68 kcal/100 g dw for *Russula olivacea* (Schaeff.) Fr. and *Armillaria mellea* (Vahl) P. Kumm species, respectively. Considerable differences in the nutritional composition have been reported, not only among species but also within the same species from different origins. The differences

found could be partly due to different stages of fruit body development (Kalač 2013) as well as environmental factors that could affect the abundance of certain compounds, but the reason(s) for the variations in the composition of mushroom species collected from background areas remains unclear (Falandysz *et al.* 2007). Total energetic value has been calculated according to Regulation (EC) No. 1169/2011 of the European Parliament on the provision of food information to consumers:

$$\text{Energy}\left(\text{kcal}/100\,\text{g}\right)=4\times\left(\text{g protein}+\text{g total available carbohydrate}\right)$$
$$+2\times\left(\text{g dietary fiber}\right)+9\times\left(\text{g fat}\right).$$

3.2.5 Ash and Mineral Elements

Ash content in wild species is more variable than in cultivated ones, probably due to the diversity of substrates. However, this variability seems to be lower than for proteins, carbohydrates, and lipid content (Kalač 2013).

The amount of ash in wild mushrooms can vary between 5.53 and 37.78 g/100 g dw according to what was recorded in *Boletus edulis* Bull. and *Russula olivacea* (Schaeff.) Fr. by Heleno *et al.* (2011) and Grangeia *et al.* (2011), respectively (see Table 3.4).

Wild edible mushrooms can accumulate high quantities of macro- as well as microelements, which are essential for mushroom development and also for human health. Potassium and phosphorus are usually the predominant elements, followed by calcium, magnesium, sodium, and iron (Okoro & Achuba 2012). Potassium is unevenly distributed within the fructiferous bodies, being more abundant in the cap and less abundant in the spores (Kalač 2013). Usually ash and particularly phosphorus and potassium content is somewhat higher than in most vegetables (Kalač 2013). Table 3.7 shows macro- and microelement content for different wild edible mushroom species.

Table 3.7 Composition of macro- and microelements (mg/kg dry weight) in different wild edible mushroom species.

Species	Macroelements				Microelements				References
	Na	K	P	Ca	Mg	Fe	Mn	Zn	Cu	
Coprinus comatus (O.F. Müll.) Pers.	0.873	6.755	4.941	0.096	1.773	0.688	0.0229	0.0510	0.00456	Tel *et al.* 2014
Lepista nuda (Bull.) Cooke	0.620	21.2	2.780	8.800	3.410	0.842	0.0589	0.0755	0.0266	Gençcelep *et al.* 2009
Morchella esculenta (L.) Pers.	0.180	23.5	3.49	0.85	1.81	0.195	0.0626	0.0989	0.0547	Gençcelep *et al.* 2009
Pleurotus tuber-regium (Fr.)	0.005	0.0074	0.0046	0.0003	0.0009	0.0024	0.00006	0.0001	0.0004	Obodai *et al.* 2014
Suillus granulatus (L.) Roussel	0.344	7.250	3.091	0.120	8.132	6.028	0.228	0.354	0.002	Tel *et al.* 2014
Suillus granulatus (L.) Roussel	0.15	29.1	4.49	0.46	2.74	0.458	0.030	0.169	0.107	Gençcelep *et al.* 2009
Termitomyces robustus (Beeli) R. Heim	0.002	0.020	0.004	0.0004	0.0007	0.002	0.0007	0.0001	0.0005	Obodai *et al.* 2014
Tricholoma terreum (Schaeff.) P. Kumm.	1.061	3.333	1.655	7.210	9.910	6.110	0.030	0.042	0.002	Tel *et al.* 2014

Different authors such as Ayodele and Odogbili (2010), Aloupi *et al*. (2012), and Kalač (2010) point out the presence of heavy metals such as lead, cadmium, mercury, nickel, and chrome, whose consumption may produce toxicological effects in humans. Mushrooms can accumulate heavy metals whose levels will depend on species, substrate composition, and environmental factors (Kalač & Svoboda 2000). However, details on toxicological risk and nutritional evaluation of such substances are limited in mushrooms.

3.3 Vitamins

Mushrooms contain different B-complex vitamins, such as thiamine (B_1), riboflavin (B_2), and niacin (B_3). They also contain chemical compounds with antioxidant properties such as ergosterol (vitamin D precursor), β-carotene (provitamin A precursor), tocopherols (vitamin E), and ascorbic acid (vitamin C) (Cheung 2010; Heleno *et al*. 2012; Kalač 2013). For several species, the content range of thiamine was 0.02–1.6 mg/100 g dw, riboflavin 0.3–4.5 mg/100 g dw, niacin 1.2–6.6 mg/100 g dw, and ascorbic acid 1.3–2.7 mg/100 g dw (Quan *et al*. 2007; Wu *et al*. 2005; Yin & Zhou 2008; Zhou & Yin 2008).

Ergosterol turns into viosterol under ultraviolet light, and then into ergocalciferol, which is a form of vitamin D. Ergosterol is a cell membrane component in mushrooms, and fulfills the same function as cholesterol in animal cells. A relatively high content of ergosterol could be important for people who have a limited intake of cholecalciferol or vitamin D, for example vegans and vegetarians (Kalač 2013). Ergosterol was the most abundant sterol found in wild *Cantharellus cibarius* Fr. and *Boletus edulis* Bull., with 0.17–0.35 g/100 g dw (Teichmann *et al*. 2007). Mattila *et al*. (2002) found that ergosterol content was higher in cultivated mushrooms (0.60–0.68 g/100 g dw) than in wild *Cantharellus cibarius*, *Cantharellus tubaeformis* Fr., *Boletus edulis*, and *Lactarius trivialis* (Fr.) Fr. (0.29–0.49 g/100 g dw) and was similar to levels reported in Huang *et al*. (1985) and Koyama *et al*. (1984).

β-Carotene is a provitamin A precursor with antioxidant properties, which participate in free radical inhibition, thus preventing cell aging. This compound has been detected in variable amounts in wild mushrooms, *Agaricus campestris* and *A. comtulus* Fries presenting 0.6 and 0.7 mg/100 g dw, respectively, while *Clitocybe costata* Kühner & Romagn. yielded 0.07 mg/100 g dw, according to Pereira *et al*. (2012).

Tocopherols are one of the most widely studied vitamin groups; they protect the human body from effects related to oxidative stress such as cardiovascular diseases and cancer, due to their capacity to eliminate free radicals (Ferreira *et al*. 2010). Cultivated species generally present lower total tocopherol content than wild species (Kalač 2013). Moreover, total tocopherol content varies with each wild fungi species. High levels of total tocopherols have been detected in *Suillus luteus* (O.F. Müll.) Pers. (0.45 mg/100 g dw), *Cortinarius violaceus* (L.) Gray (0.35 mg/100 g dw), and *Coprinus comatus* (O.F. Müll.) Pers. (0.30 mg/100 g dw) (Reis *et al*. 2011; Vaz *et al*. 2011), while *Lepista sordida* (Schumach.) Singer had a very low total tocopherol content (0.002 mg/100 g dw; Heleno *et al*. 2010).

Low levels of ascorbic acid are present in different species. Values ranging between 0.66 and 33.16 mg/100 g dw in *Hygrophorus chrysodon* (Batsch) Fr. and *Ramaria aurea* (Schaeff.) Quél. have been reported (Pereira *et al*. 2012).

3.4 Conclusion

Fungi species described in this chapter are, in some cases, widely used and consumed by people from different regions of the world.

The available data summarized in this chapter indicate that wild edible mushrooms constitute an excellent nutrient source for humans, especially in low-caloric diets due to their low fat content and energetic value, and suitable for people with high cholesterol levels. This is thought to be due to the diversity of unsaturated fatty acids, relevant for metabolic pathways and human health. In addition to this, mushrooms are rich in proteins, amino acids,

carbohydrates, dietary fiber, minerals, and vitamins. According to the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans (USDA 2010), a 2000 calorie diet should contain 90 g of crude protein daily, and a 100 g portion of dry wild mushrooms could provide between 13.33% and 66% that (Barros *et al*. 2008). The recommended carbohydrate amount is 260 g daily, and a 100 g portion of dry mycorrhiza mushrooms contributes around 6.15–16.15% of the daily requirement, while 100 g of dry saprotrophic mushrooms contributes 0.15–5.76% of the daily requirement (Grangeia *et al*. 2011). The recommended dietary fiber amount is 30 g, and a 100 g portion of dry wild mushrooms provides 27.6–123.3% of the daily requirement (Nile & Park 2014; Sanmee *et al*. 2003). The recommended amount of lipids is 71 g, and 100 g of dry wild mushrooms provides 0.15–11.29% of the daily requirement (Akata *et al*. 2012; Pereira *et al*. 2012). Regarding vitamins, the daily recommended amounts are thiamine 1.8 g, riboflavin 2.2 mg, niacin 23 mg, and ascorbic acid 126 mg. A 100 g portion of dried wild mushrooms can provide 1.11–88.88% of the daily thiamine required (Quan *et al*. 2007), 13.63–204.4% of the daily riboflavin requirement (Wu *et al*. 2005), 5.21–28.69% of the daily niacin requirement (Yin & Zhou 2008), and 1.03–2.14% of the daily ascorbic acid requirement (Zhou & Yin 2008).

There is great variability in the intraspecific nutritional values reported for wild mushroom species compared to cultivated ones. This is related to the possibility of manipulating and standardizing different stages during production processes. The possibility of genetically selecting particular strains, using different additives in growth substrates, which allow for improving and homogenizing nutrient content, as well as manipulating certain environmental conditions such as light, humidity, and temperature allow for decreasing variability and manipulating concentrations. Even though several studies on the effects of using irradiation techniques on nutrient composition have been recently published (Fernandes *et al*. 2012, 2013), future research should elucidate aspects such as processing effects on nutrient contents, as well as nutrient bioavailability.

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4
The Bioactive Properties of Mushrooms

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4.1 Introduction

In recent years, interest has been growing in the mechanisms of action of medicinal mushrooms. For over 1000 years, mushrooms have been used in folk medicine in Asia to prevent and cure a multitude of quite different diseases. The most well-known examples are *Ganoderma lucidum* (Curtis) P. Karst, *Phellinus linteus* (Berk. & M. A. Curtis) Teng, *Cordyceps sinensis* (Berk.) Sacc., *Trametes versicolor* (L.) Lloyd, and *Inonotus obliquus* (Ach. ex Pers.) Pilát.

Mushrooms have been consumed extensively in humans' daily diet as a supplementary food item since ancient times. Nowadays there is increasing public interest in secondary metabolites from mushrooms which may allow the synthesis of new drugs. Mushrooms are an excellent source of secondary metabolites, vitamins, minerals, protein, and carbohydrates, as well as being high in fiber and low in fat. They also contain various bioactive molecules, including terpenoids, steroids, phenols, nucleotides, glycoprotein derivatives, and polysaccharides. Therefore, they have been considered as a potential source of antioxidant, antitumor, antiviral, antimicrobial, and immunomodulatory agents. They have been shown to modulate the immune system and to have hypoglycemic, antithrombotic, antibiotic, antitumor, antiviral, antihypertensive, and antilipidemic properties, as well as inflammation inhibition and antimicrobial action (Alves *et al*. 2013; Popovic *et al*. 2013). Researchers are particularly seeking novel prototype therapeutic agents representing new chemical

classes, operating by different modes of action compared to the existing agents and, consequently, lacking cross-resistance to chemicals currently used.

The fungal kingdom possesses certain natural advantages in terms of dietary importance over the rest of the vegetarian platter. These are: (a) a good protein content (20–30% of dry matter) with all the essential amino acids (yeasts are especially enriched in lysine), thus being capable of substituting for meat, (b) chitinous wall to act as a source of dietary fiber, (c) high vitamin B content, and (d) low in fat.

Mushrooms have been used not only as a source of food but as a medicinal resource as well. The medicinal properties of mushrooms have been confirmed through intensive research conducted worldwide. Medicinal mushrooms have been used as a dietary supplement or medicinal food in China for over 2000 years. The extractable ingredients of mushrooms have been incorporated into other products and have been claimed to improve the biological function of the human body. Fungi from the Basidiomycota have attracted interest because they contain a large number of biologically active elements such as polysaccharides, sterols, and phenolic compounds.

4.2 Antimicrobial Activity of Edible and Medicinal Fungi

Mushrooms need antibacterial and antifungal compounds to survive in their natural environment and therefore it is not surprising that antimicrobial compounds with more or less strong activities can be isolated from many mushrooms and that they could be of benefit for humans (Lindequist *et al*. 1990).

4.2.1 Antibacterial Activity of Mushroom Extracts

According to the World Health Organization (WHO 2014), the bacterial infections which contribute most to human disease are also those in which emerging and microbial resistance is most

evident, such as diarrheal diseases, respiratory tract infections, meningitis, sexually transmitted infections, and hospital-acquired (nosocomial) infections. The following are some common examples of resistant bacteria species: penicillin-resistant *Streptococcus pneumoniae*, vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, and multiresistant salmonellae and *Mycobacterium tuberculosis*. With the recent emergence of the resistant *E. coli*-linked NDM-1 "superbug," there is an urgent need to combat pathogens. Antimicrobial resistance in both medicine and agriculture is now a glaring reality. It represents a significant challenge of global dimensions to human and veterinary medicine with the prospect of therapeutic failure for life-saving treatments (see The Copenhagen Recommendations: Report from the Invitational EU Conference on The Microbial Threat: http://soapimg.icecube.snowfall.se/strama/Kopenhamnsmotet_1998.pdf).

Table 4.1 details the mushroom species possessing antibacterial activities, samples, type of extract, assays applied, and bacterial species investigated, as well as the numerical values of the results.

Table 4.1 Antibacterial activity of mushroom extracts.

Mushroom	**Origin**	**Extracts**	**Antimicrobial activity assay/ results**	**Microorganisms used**	**Reference**
Agaricus bisporus (J.E. Lange) Imbach	Netherlands (cultivated)	Methanol Ethanol	Microdilution method MIC 0.3–1.15 mg/mL MBC 0.6–4.7 mg/mL MIC 0.03–3.6 mg/mL MBC	*Staphylococcus aureus, Bacillus cereus, Micrococcus flavus, Listeria monocytogenes, Escherichia coli, Salmonella typhimurium,*	(Stojkovic *et al.* 2014a)

				0.06–4.7 mg/mL	*Pseudomonas aeruginosa, Enterobacter cloacae*	
A. bisporus	Portugal (wild)	–	Methanol	Agar diffusion method MIC 5.0– µg/mL	*Bacillus subtilis*	(Barros *et al*. 2008a)
A. bisporus	Turkey (wild)		Methanol	Agar diffusion method Inhibition zone 19–22 mm at 200 µg/ disk	*B. cereus, M. flavus, Micrococcus luteus, Staphylococcus epidermidis, S. aureus*	(Öztürk *et al*. 2011; Ozen *et al*. 2011; Tambekar *et al*. 2006)
A. bisporus	Nigeria (wild)		Methanol	Agar diffusion method Inhibition zone 4.33–9.00 at 100 µg/ mL	*P. aeruginosa, E. coli, S. typhimurium, Shigella flexneri, B. cereus, L. monocytogenes*	(Abah & Abah 2010)
A. brasiliensis Wasser, Didukh, Amazonas & Stamets	Netherlands (M7700, mycelia)		Methanol Ethanol	Microdilution method MIC 0.1–2.3 mg/ mL MBC 0.3–4.6 mg/mL MIC 0.04–0.35 mg/	*S. aureus, B. cereus, M. flavus,L. monocytogenes, E. coli, S. typhimurium, P. aeruginosa, E. cloacae*	(Stojkovic *et al*. 2014a)

			mL MBC 0.08–1.5 mg/mL		
A. brasiliensis	Brazil (wild)	Ethanol	Microdilution method MIC-MBC 0.08–0.44 mg/mL	*Staphylococcus mutans*, *S. sobrinus*	(Lund *et al*. 2009)
A. cf. *nigrecentulus* Heinem	Brazil (wild)	– Ethyl acetate	Agar diffusion method MIC 10 µg/mL	*Streptococcus saprophyticus*	(Rosa *et al*. 2003)
Agrocybe aegerita (V. Brig.) Singer	Serbia (wild)	Methanol	Microdilution method MIC 0.59–4.74 mg/mL MBC 1.18–9.49 mg/mL	*S. aureus*, *B. cereus*, *M. flavus*, *L. monocytogenes*, *E. coli*, *S. typhimurium*, *P. aeruginosa*, *E. cloacae*	(Petrovic *et al*. 2014a)
Boletus aereus Bull.	Serbia (wild)	Methanol	Microdilution method MIC 0.40–3.0 mg/mL MBC 1.50–6.0 mg/mL	*S. aureus*, *B. cereus*, *M. flavus*, *L. monocytogenes*, *E. coli*, *S. typhimurium*, *P. aeruginosa*, *E.*	(Stojkovic *et al*. 2013c)

				cloacae, P. aeruginosa	
B.edulis Bull.	Portugal (wild) Spain (cultivated)	Methanol Methanol Water	Agar diffusion method MIC 5 µg/mL Microdilution method MBC 8.0–9.5 mg/mL MBC 3.8–7.8 mg/mL	*S. aureus* *S. aureus, E. coli*	(Barros *et al.* 2008a) (Santoyo *et al.* 2009)
B. lupinus Fries	Macedonia (wild)	Methanol	Agar diffusion method MIC 6.25–50 mg/mL	*Bacillus subtilis, B. pumilus, Staphylococcus lutea, S. aureus, P. aeruginosa*	(Nedelkoska *et al.* 2013)
Clitocybe alexandri (Fr.) Staude	Turkey (wild)	Methanol Ethanol Ethyl acetate *n*-Hexane Water	Agar diffusion method Inhibition zone 9–26 mm at 100 µg/disk	*B. cereus, B. subtilis, S. aureus, E. coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas fluorescens, M. luteus, Enterobacter*	(Solak *et al.* 2006)

				aerogenes, *S. typhimirium*, *Serratia marcescens*	
Coprinopsis atramentaria (Bull.) Redhead, Vilgalys & Moncalvo	Portugal (wild) Nigeria (cultivated)	Methanol Methanol, hexane, chloroform, ethyl acetate	Microdilution method MIC 0.40–3.0 mg/mL MBC 1.50–6.0 mg/mL Microdilution method MIC 0.40–3.0 mg/mL MBC 1.50–6.0 mg/mL	*S. aureus*, *B. cereus*, *M. flavus*, *L. monocytogenes*, *E. coli*, *S. typhimurium*, *P. aeruginosa*, *E. cloacae* *E. coli*, *P. aeruginosa*, *S. typhi*, *S. aureus*, *M. luteus*, *B. subtilis*	(Heleno *et al*. 2014) (Osuji *et al*. 2013)
Coprinus comatus (O.F. Mull.) Gray	Netherlands (cultivated) Serbia (wild) Sudan (wild)	Methanol Ethanol Petroleum ether	Microdilution method MIC 0.0625–3.0 mg/mL MBC 0.125–6.25 mg/mL Agar diffusion method MIC 2.5–5.0 mg/	*S. aureus*, *B. cereus*, *M. flavus*, *L. monocytogenes*, *E. coli*, *S. typhimurium*, *P. aeruginosa*, *E. cloacae* *S. aureus*, *E. coli*	(Stojkovic *et al*. 2013a) (Ehssan & Saadabi 2012; Johansson *et al*. 2001)

			mL		
Cordyceps militaris (L.: Fr.) Link	Korea (cultivated) India (wild)	Methanol Methanol Water	Microdilution method MIC 0.015–3.0 mg/mL MBC 0.03–6.25 mg/mL Agar well diffusion method Inhibition 17.64–25.09%	*S. aureus, B. cereus, M. flavus, L. monocytogenes, E. coli, S. typhimurium, P. aeruginosa, E. cloacae* *S. aureus, E. coli*	(Reis *et al*. 2013) (Pathania & Sagar 2014)
Ganoderma lucidum (Curtis) P. Karst *G. lucidum*	Portugal (wild) Serbia (wild) China (cultivated)	Methanol Methanol	Microdilution method MIC 0.0125–0.75 mg/mL MBC 0.035–1.5 mg/mL Microdilution method MIC 0.017–0.15 mg/mL MBC 0.035–0.30 mg/mL	*S. aureus, B. cereus, M. flavus, L. monocytogenes, E. coli, S. typhimurium, P. aeruginosa, E. cloacae* *S. aureus, B. cereus, M. flavus, L. monocytogenes, E. coli, S. typhimurium, P. aeruginosa,*	(Heleno *et al*. 2013) (Stojkovic *et al*. 2014b)

			MIC 0.035–0.15 mg/mL MBC 0.07–0.30 mg/mL	*E. cloacae*	
G. praelongum Murrill *G. resinaceum* Boud. *G. lucidum*	India (wild)	Ethyl acetate Water	Agar diffusion method Inhibition 19.50 mm Inhibition 14.29 mm Inhibition 14.14 mm No inhibition	Methicillin-sensitive *S. aureus* (MSSA) Methicillin-resistant *S. aureus* (MRSA)	(Ameri *et al.* 2011)
G. pfeifferi Bres.	Germany (wild)	Methanol	Agar diffusion method Inhibition 15.50–32 mm	*Pityrosporum ovale, Staphylococcus epidermidis, Propionibacterium acnes*	(Lindequist *et al.* 2005)
Laetiporus sulphureus (Bull.) Murrill	Serbia (wild) Turkey (wild)	Aqueous Methanol –Acetone – Dichloromethane –*n*-Hexane – Chloroform –Ethanol Methanol Ethanol	Microdilution method MIC 1.5 mg/mL MIC 0.63–20.0 mg/mL MBC 1.25–30.0 mg/mL	– – – – – – – *S. aureus, B. cereus, M. flavus, L. monocytogenes,*	(Siljegovic *et al.* 2011) – – – (Petrovic *et al.* 2013) – – –

Methanol Polysaccharide Methanol Ethanol	MIC 2.5–20.0 mg/mL MBC 5.0–30.0 mg/mL MIC 1.25–10.0 mg/mL MBC 2.5–20.0 mg/mL MIC 0.15–2.0 mg/mL MBC 0.3–4.0 mg/mL MIC 0.4–1.56 mg/mL MBC 0.78–3.125 mg/mL MIC 0.50–>2.0 mg/mL MIC 0.02–4.5 mg/mL MIC 0.4–3.1 mg/mL MBC	*E. coli, S. typhimurium, P. aeruginosa, E. cloacae* – – – – *P. aeruginosa*, *S. enteritidis*, *E. coli*, *Morganella morganii*, *Yersinia enterocolitica*, *Klebsiella pneumoniae*, *P. vulgaris*, *S. aureus*, *M. luteus*, *M. flavus*, *B. subtilis*, *B. cereus*	– – (Sinanoglou *et al.* 2015) (Petrovic *et al.* 2014c) – – – –, – (Petrovic *et al.* 2014b; Turkoglu *et al.* 2007)

			2.4–6.2 mg/mL MIC 0.9–3.6 mg/mL MBC 1.8–7.2 mg/mL Agar diffusion method Inhibition zone 5.0–23.0 mm		
Lentinula edodes (Berk.) Pegler	UK (cultivated)	Aqueous	Agar diffusion method Inhibition zone 8–92 mm	*B. cereus, B. subtilis, B. pumilis, Cupriavidis* spp., *E. coli, Enterococcus faecalis, Klebsiella aerogenes, K. pneumoniae, L. monocytogenes, P. aeruginosa, Pseudomonas* spp., *Salmonella poona, Serratia*	(Hearst *et al.* 2009)

				marcescens, S. aureus (MSSA), S. epidermidis, Staphylococcus spp.	
Lycoperdon pusilum Batsch *L. giganteum* Batsch	Nigeria (wild)	Methanol Ethanol Water	Agar well diffusion methods Inhibition zone 4.0–19.0 mm Inhibition zone 5.0–17.0 mm	*B. cereus, E. coli, K. pneumoniae, Proteus vulgaris, P. aeruginosa, S. aureus*	(Jonathan & Fasidi 2003)
Morchella conica Pers.	Turkey (wild)	Ethanol	Agar diffusion methods Inhibition zone 4.0–29.0 mm	*P. aeruginosa, S. enteritidis, E. coli, Morganella morganii, Yersinia enterocolitica, K. pneumoniae, P. vulgaris, S. aureus, M. luteus, M. flavus, B. subtilis, B. cereus*	(Turkoglu *et al.* 2006)
M. esculenta	Portugal (wild)	– –	Agar well diffusion	*S. aureus, B. cereus*	(Stojkovic *et al.*

(L.) Pers.	Serbia (wild) Portugal (wild) Serbia (wild)	Methanol	methods Inhibition zone 6.16–7.80 mm Inhibition zone 6.18–8.34 mm Microdilution method MIC 0.3–>10.0 mg/mL MBC 0.6–>10.0 mg/mL MIC 0.02–1.25 mg/mL MBC 0.05–1.25 mg/mL	*M. flavus, L. monocytogenes, E. coli, S. typhimurium, P. aeruginosa, E. cloacae*	2013c)
Phellinus linteus (Berk. & Curtis) Teng	Thailand (wild)	Methanol Ethanol Polysaccharides Glucans Triterpenoids	Microdilution method MIC 0.032–2.2 mg/mL MBC 0.043–2.7 mg/mL	*S. aureus, B. cereus, M. flavus, L. monocytogenes, E. coli, S. typhimurium, P. aeruginosa, E. cloacae*	(Reis *et al.* 2014a)

Phellinus igniarius (L.) Quel.	Macedonia (wild)	Methanol	Agar diffusion method MIC 2.5–10.0 mg/mL	*B. subtilis, Bacillus pumilus, S. aureus, Staphylococcus lutea, P. aeruginosa*	(Nedelkoska *et al.* 2013)
Pleurotus ostreatus (Jacq. ex Fr.) Kumer	UK (cultivated)	Aqueous	Agar diffusion method Inhibition zone 5.0–20.0 mm	*B. cereus, B. subtilis, P. aeruginosa*	(Hearst *et al.* 2009)
P. sajor-caju (Fr.) Singer	India (cultivated)	Aqueous Organic solvents	Agar diffusion method Inhibition zone 12.0–20.0 mm Inhibition zone 12.0–19.0 mm	*Enterobacter aerogenes, E. coli, K. pneumoniae, P. vulgaris, P. aeruginosa, S. typhimurium, S. aureus*	(Tambekar *et al.* 2006)
Suillus granulatus (L.) Roussel	Portugal (wild) Serbia (wild)	– – Methanol	Microdilution method MIC 0.1–0.2 mg/mL MBC 0.2–0.4 mg/mL MIC 0.04–0.15 mg/mL	*S. aureus, B. cereus, M. flavus, L. monocytogenes, E. coli, S. typhimurium, P. aeruginosa, E. cloacae*	(Reis *et al.* 2014b)

			MBC 0.05–0.2 mg/mL		
Tirmania pinoyi (Maire) Malencon	Libya (wild) Algeria (wild)	Water Methanol DMSO Ethyl acetate	Microdilution method MIC 1.5–2.0 mg/mL MBC 2.0–2.5 mg/mL Agar diffusion method Inhibition zone 6.0–22.0 mm	*S. aureus*, *B. cereus*, *M. flavus*, *L. monocytogenes*, *E. coli*, *S. typhimurium*, *P. aeruginosa*, *E. cloacae*, *B. subtilis*, *Enterobacter* sp. *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *Enterobacter* sp.	(Stojkovic *et al*. 2013b) Dib-Bellahouel & Fortas 2011)

DMSO, dimethylsulfoxide; MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration.

During recent decades, several pathogenic microorganisms have developed resistance to available antibiotics. Infections by multidrug-resistant isolates of *Staphylococcus epidermidis*, *S. aureus*, *Streptococcus* spp., *Enterococcus* spp., and *Escherichia coli*, among others, have become more and more frequent, stimulating the search for new antibiotics with novel mechanisms of action.

The best antibacterial activity was observed for *Ganoderma luciudm* and *Coprinus comatus* methanolic extracts.

4.2.2 Compounds Isolated from Mushrooms as Bacterial Growth Inhibitors

Of special interest are compounds with activities against multiresistant bacterial strains. Agrocybin, a compound able to halt the growth of Gram-positive, Gram-negative and acid-fast bacteria, was isolated from *Agrocybe dura* (Bolton) Singer (Kavanagh *et al*. 1950). Its activity against *Bacillus mycoides*, *B. subtilis*, *E. coli*, *Klebisiella pneumoniae*, *Mycobacterium pheli, M. smegmatis*, *Photobacterium fischeri*, *Pseudomonas aeruginosa*, and *S. aureus* was demonstrated. Berg *et al*. (2002) report the isolation of agrocybolacton from *Agrocybe* spp. This compound shows moderate antibacterial activity against Gram-positive bacteria such as *B. subtilis* and *M. smegmatis* at concentrations near 50 μg/mL.

Coprinol, a new antibacterial cuparane-type terpenoid from cultures of a *Coprinus* sp., exhibited activity against multidrug-resistant Gram-positive bacteria (Johansson *et al*. 2001). Micaceol, a sterol, and (Z,Z)-4-oxo-2,5-heptadienedioic acid were isolated from *Coprinus* (currently valid name *Coprinopsis micaceus*) with activities against *Corynebacterium xerosis* and *S. aureus* (Zahid *et al*. 2006).

The main active constituent of *Cordyceps militaris* (L.) Fr. fruiting bodies is cordycepin, a derivative of the nucleoside adenosine. This molecule was first isolated from *C. militaris* (Cunningham *et al*. 1951) and it is now produced synthetically as it has antibacterial properties (Paterson & Russel 2008).

An antibacterial hirsutane sesquiterpene, coriolin, was isolated from the white-rot basidiomycete *Coriolus consors* (Berk.) Imazeki, being active against *S. aureus*, *Micrococcus flavus*, *B. subtilis*, and *B. anthracis* with the same minimum inhibitory concentration (MIC) values of 12.5 μg/mL (Takeuchi *et al*. 1969).

Several lanostanoid derivatives, polyporenic acid C, 3R-acetyloxylanosta-8,24-dien-21-oic acid, pinicolic acid A, trametenolic acid B, and fomitopsic acid, isolated from the

polypore *Fomitopsis pinicola* (Swartz: Fries) Karst. have shown antimicrobial activity against *B. subtilis* in a TLC-bioautography assay in quantities from 0.01 to 1 µg, but did not inhibit *B. subtilis* in a classic agar dilution assay at concentrations up to 50 µg/mL (Keller *et al*. 1996).

Three sterols: 5α-ergost-7en-3β-ol, 5α-ergost-7,22-dien-3β-ol, and 5,8-epidioxy-5α,8α-ergost-6,22-dien-3β-ol, as well as a novel lanostanoid were isolated from *Ganoderma applanatum* (Pers.) Pat. (Smania *et al*. 1999). The antibacterial activity of these compounds was determined by MIC and minimum bactericidal concentration (MBC). Among the seven bacterial species tested, the Gram-positives (*B. cereus*, *Corynebacterium diphtheriae*, *Staphylococcus saprophyticus*, *S. aureus*, and *S. pyogenes*) were more sensitive (MIC 0.003–2.0 mg/mL; MBC 0.06–4.0 mg/mL) than the Gram-negatives (*E. coli* and *P. aeruginosa*, MIC 1.0–4.0 mg/mL; MBC 2.0–4.0 mg/mL). Among the novel lanostane triterpenoids, ganorbiformins A–G, isolated from *Ganoderma orbiforme* (Fr.) Ryvarden, the C-3 epimer of ganoderic acid T, also exhibited significant antimycobacterial activity with a MIC value of 1.3 µM (Isaka *et al*. 2013). The new sesquiterpenoid hydroquinones produced by *Ganoderma pfeifferi* Bres., named ganomycins A and B, inhibit the growth of methicillin-resistant *S. aureus* and other bacteria (Smania *et al*. 2003).

Liu *et al*. (2010) isolated novel compounds with effective antimicrobials from two American mushroom species, *Jahnoporus hirtus* (Quell.ex.Cke.) Nuss. and *Albatrellus flettii* Morse ex Pouzar: 3,11-dioxolanosta-8,24(Z)-diene-26-oic acid, a new lanostane-type triterpene, from *J. hirtus* and confluentin, grifolin, and neogrifolin from *A. flettii*. Grifolin showed promising activity against *B. cereus* (10 µg/mL) and *Enterococcus faecalis* (0.5 µg/mL).

Lentinan from the shiitake mushroom (*Lentinula edodes* (Berk.) Pegler) inhibited *M. tuberculosis* and *L. monocytogenes* (Chihara 1992). Oxalic acid is one agent responsible for the antimicrobial effect of *L. edodes* against *S. aureus* and other

bacteria (Bender *et al*. 2003).

The merulinic acids A, B, and C isolated from the fruiting bodies of the polypore *Merulius tremellosus* Schrad. showed antimicrobial activity with MIC values of 0.4–10 µg/mL, particularly against *Arthrobacter citreus*, *B. subtilis*, *Corynebacterium insidiosum*, *Micrococcus roseus*, and *Sarcina lutea*. *Staphylococcus aureus* and *Proteus vulgaris* were inhibited only by merulinic acid B (Stamets 2001).

Scorodonin, a biologically active metabolite from *Marasmius scorodonius* (Fr.: Fr.) Fries, inhibits Gram-negative and Gram-positive bacteria (Anke *et al*. 1980). Marasmic acid was shown to be an antibacterial, antifungal, cytotoxic, phytotoxic substance isolated from *M. conigenus* Rea (Abraham 2001).

One of the first antimicrobial compounds ever isolated from a polypore was biformin, a polyacetylenic carbinol. Biformin is produced by *Trichaptum biforme* (Fr.) Ryvarden (as *Polyporus biformis*) and is active against a wide variety of bacteria and fungi (Robbins *et al*. 1947). Plectasin peptide, obtained from *Pseudoplectania nigrella* (Persoon) Fuckel, is the isolated compound with the highest antimicrobial activity against Gram-positive bacteria, while 2-aminoquinoline, isolated from *Leucopaxillus albissimus*, presents the highest antimicrobial activity against Gram-negative bacteria (Alves *et al*. 2012).

The antimicrobial activity of *Pycnoporus sanguineus* (L.) Murrill has been known since 1946, when Bose (1946) isolated poliporin, a compound active against Gram-positive and Gram-negative bacteria and without toxicity, using animal experiments. More recently, studies by Smania *et al*. (1997) showed that this basidiomycete produces cinnabarin, a phenoxazinone with an orange pigment active against Gram-positive and Gram-negative bacteria. The red polypore *P. sanguineus* also produces cinnabarin, with *B. cereus* and *Leuconostoc plantarum* being the most sensitive, presenting a MIC value of 62.5 µg/mL (Smania *et al*. 1998). Novel butenolides, ramariolides A–D, isolated from the fruiting bodies of the coral mushroom *Ramaria cystidiophora* (Kauffman) Corner, showed *in vitro* antimicrobial

activity against *Mycobacterium smegmatis* and *M. tuberculosis* (Centko *et al*. 2012). Two hirsutane derivatives, hirsutic acid and complicatic acid, were isolated from the wood-decaying polypore *Stereum complicatum* (Schwein.) Burt (Mellows *et al*. 1973). Similar to other hirsutanes with α-unsaturated exomethylene ketone system, complicatic acid showed moderate antimicrobial activity against *S. aureus* (Mantle & Mellows 1973).

The aromatic acetylene derivatives frustulosin and frustulosinol isolated from liquid cultures of *S. frustulosum* were active against several bacteria such as *S. aureus*, *B. mycoides*, and *B. subtilis* and also moderately active against *Vibrio cholerae* and *V. cholerae* phage (Nair & Anchel 1977). Coloratin A [3,5-dimethoxy-2-(6-oxo-5-pentyl-6H-pyran-3-carbonyl)-benzoic acid] and coloratin B (2-carbomethoxyl-3,5-dimethoxybenzoic acid) extracted from *Xylaria intracolorata* J.D. Rogers, Callan & Samuels had reasonable antimicrobial activity against several microbes (Quang *et al*. 2006).

4.2.3 Antifungal Activity of Crude Mushroom Extracts

Fungal infections pose a continuous and serious threat to human health and in recent years there has been an increased use of antifungal agents which has resulted in the development of resistance, toxicity, and low efficacy rates. This has given rise to the search for new natural antifungal agents. Macrofungi seem promising in terms of compounds with potential biological activities. In recent decades, interesting compounds of different biogenetic origins have been isolated from Basidiomycota and found to have antifungal activity. The chemical composition and the potential for antifungal activity depend highly on the fungal species, habitat, phase of life cycle (mycelium, young or mature fruiting body), method of processing, extraction solvent, and extract dose.

A total of 103 isolates of basidiomycetes, representing 84 species from different Brazilian ecosystems, were used in a bioassay panel (Rosa *et al*. 2003). Furthermore, Alves *et al*. (2013) also gave a

comprehensive overview of mushroom extracts and compounds with antifungal activity.

Moreover, our research group has done extensive work on the antifungal characterization of wild and cultivated mushroom species. The antifungal potential of extracts of wild macromycetes (*Agaricus albertii*, *Agrocybe aegerita*, *Boletus aereus*, *Calocybe gambosa*, *Coprinus comatus*, *Ganoderma lucidum*, *Morchella esculenta*, *M. conica*, *Suillus granulatus*, *Tirmania pinoyi*, *and Phellinus linteus*) and cultivated mushroom (*Agaricus bisporus*, *A. brasiliensis*, *Coprinus comatus*, *Cordyceps militaris*, and *Ganoderma lucidum*) have been evaluated *in vitro* and *in situ*. *In vitro* antimicrobial activity has been investigated by the microdilution method, using a panel of pathogenic microfungi, and by testing mushroom extracts obtained from different extraction solvents (Petrovic *et al*. 2013, 2014a,b,c; Reis *et al*. 2012a, 2013, 2014a,b; Stojkovic *et al*. 2013a,b,c, 2014a,b). The enormous structural diversity of natural compounds originating from mushrooms offers prospective potential for the discovery of new drugs and wild mushroom species are s possible source of bioactive compounds. In the study presented by Alves *et al*. (2013), different compounds isolated from mushrooms with antifungal activity are reported.

In fact, there is a gap in the identification of individual compounds responsible for antifungal properties, and only a few low molecular weight compounds, some peptides, and proteins have been described.

4.2.4 Isolated Compounds from Mushrooms Express Antifungal Potency

Most studies on mushrooms with antifungal activity describe the action of their extracts without identifying the compounds responsible for this activity. However, some low molecular weight (LMW) and high molecular weight (HMW) compounds have been described as active against microfungi.

The LMW terpene compound grifolin seems to have the highest antifungal activity (Rosa *et al*. 2003), but other LMW compounds

also showed some activity (e.g. rufuslactone, enokipodim F, G, I, cloratin A, and 2-aminoquinoline). The sesquiterpene rufuslactone showed activity against some phytopathogenic fungi such as *Alternaria alternata*, *A. brassicae*, *Botrytis cinerea*, and *Fusarium graminearum*. Furthermore, the growth inhibition percentage of this compound in *A. alternata* (38.9%) was higher than that obtained for the positive control, carbendazim (~10%) (Luo *et al*. 2005). Other sesquiterpenes, enokipodim F, G and I, isolated from *Flammulina velutipes* (Curtis) Singer mycelium presented low activity against *Aspergillus fumigatus* with IC_{50} values of 229.1, 233.4 and 235.1 μM, respectively (Wang *et al*. 2012).

Phenolic acids and related compounds such as *p*-hydroxybenzoic and cinnamic acids identified in *Ganoderma lucidum* also revealed activity against different fungi species, such as *Aspergillus fumigatus*, *A. versicolor*, *A. ochraceus*, *A. niger*, *Trichoderma viride*, *Penicillium funiculosum*, *P. ochrochloron*, and *P. verrucosum* (with MICs of 0.003–0.12 mg/mL and 0.007–0.03 mg/mL). Moreover, these compounds gave higher activity than the standards, bifonazole (MIC 0.15 mg/mL) and ketoconazole (MIC 1.0 mg/mL) (Heleno 2013). Cloratin A, a derivative of benzoic acid, was isolated from *Xylaria intracolarata* and showed activity against *Aspergillus niger* (inhibition zone diameter (IZD) 15 mm) and *Candida albicans* (IZD 17 mm), similar to the control (nystatin, IZD 17 mm) (Quang *et al*. 2006). Smania *et al*. (2007) reported a reduced activity of two LMW compounds isolated from *Ganoderma australe* (Fr.) Pat. (australic acid and methyl australate) against *Candida albicans*, *Microsporum canis*, and *Trichophyton mentagrophytes*. Australic acid proved to be more active against filamentous fungi.

Chrysotriones A and B, two acylcyclopentenediones isolated from *Hygrophorus chrysodon* (Batsch) Fr., exhibited activity against *Fusarium verticillioides* (Gilardoni *et al*. 2007).

Three steroids (5α-ergost-7-en-3β-ol, 5α-ergost-7,22-dien-3β-ol, and 5,8-epidioxy-5α,8α-ergosta-6,22-dien-3β-ol) and five terpenes

(applanoxidic acid A, C, F, G, and H), isolated from *Ganoderma annulare* (Fr.) Gilb., revealed activity against *Microsporum canis* and *Trichophyton mentagrophytes*.

Applanoxidic acid A showed the best activity against the mentioned fungi, and particularly for *Trichophyton mentagrophytes* it demonstrated higher activity (MIC 500 μg/mL) than the positive control (fluconazole; MIC 0.6 μg/mL).

According to the data obtained, antifungal activity observed for the above-mentioned compounds is not comparable to the antibiotics most commonly used for fungal diseases. Nevertheless, future studies could chemically modify these compounds in order to increase their antifungal activity (Smania *et al*. 2003). The quinolone 2-aminoquinoline, isolated from *Leucopaxillus albissimus* (Peck) Singer, has been described in several studies showing broad spectra of biological activities. Nonetheless, a weak activity of this LMW compound was reported against *Penicillium inflatum* and *Streptomyces galilaeus* and the concentration of this compound in the mushroom is 40 times higher than the one used in the assay (Pfister 1998).

High molecular weight compounds with antifungal properties have also been isolated from mushrooms. Gonodermim, an antifungal protein isolated from *Ganoderma lucidum*, has shown activity against phytopathogenic fungi such as *Botrytis cinerea* (IC_{50} 15.2 μM), *Fusarium oxysporum* (IC_{50} 12.4 μM), and *Physalospora paricola* (IC_{50} 18.1 μM). These pathogens are commonly present in food, including cotton, cucumber, and apple, respectively. Therefore, the isolation of antifungal proteins with activity upon those toxin producers' fungi might have important applications in the food industry (Wang & Ng 2006). Another antifungal protein is ribonuclease, obtained from *Pleurotus sajor-caju* (Fr.) Singer, which showed activity against *Fusarium oxysporum* and *Mycosphaerella arachidicola* (IC_{50} values 95 and 75 μM, respectively) (Ngai & Ng 2004). Trichogin, another antifungal protein isolated from the mushroom *Tricholoma giganteum* Massee, showed antifungal activity against *F. oxysporum*, *M. arachidicola*, and *Physalospora piricola* (Guo *et al*. 2005).

Eryngin, an antifungal peptide isolated from *Pleurotus eryngii* (DC.) Quél. fruiting bodies, also demonstrated activity against *F. oxysporum* and *M. arachidicola* (Wang & Ng 2004). Its N-terminal sequence showed some similarity to the antifungal protein LAP obtained from the mushroom *Lyophyllum shimeji* (Kawam.) Hongo (Lam & Ng 2001a). Lyophyllin and LAP isolated from *L. shimeji* revealed activity against *P. piricola* (Lam & Ng 2001a). Guo *et al*. (2005) reported that trichogin was significantly different from other antifungal proteins such as LAP (Lam & Ng 2001a) and eryngin (Wang & Ng 2004). Hypsin, isolated from *Hypsizigus marmoreus* (Peck) H.E. Bigelow fruiting bodies, showed activity against *Botrytis cinerea*, *Fusarium oxysporum*, *M. arachidicola*, and *P. piricola* (Lam & Ng 2001b). Lentin, isolated from *Lentinus edodes*, showed activity against *M. arachidicola* (Lam & Ng 2001b). Another peptide with antifungal activity was pleurostrin, isolated from *Pleurotus ostreatus*, which showed activity against *F. oxysporum*, *M. arachidicola*, and *P. piricola* (Chu *et al*. 2005). Agrocybin, an antifungal peptide isolated from *Agrocybe cylindracea* (DC.) Gillet, showed activity against *M. arachidicola* (Ngai *et al*. 2005). Cordimin is a peptide that inhibited the growth of *Bipolaris maydis*, *M. arachidicola*, *Rhizoctonia solani*, and *Candida albicans* (IC_{50} 50 μM, 10 μM, 80 μM, and 0.75 mM, respectively). Nevertheless, no effects were observed against *Aspergillus fumigatus*, *F. oxysporum*, and *Valsa mali* (Wong *et al*. 2011).

The mechanisms of action of most of the LMW compounds described above are not available in literature. Regarding proteins, mainly lyophyllin and hypsin, the mechanism of action involves ribosomal inactivation. The mode of action of many other proteins remains unknown but is being extensively researched (Selitrennikoff 2001). In the literature, the authors compare the studied compounds with others revealing antifungal activity. Ribonuclease presents an N-terminal sequence similar to the one present in the bacteriocine peptide of *Lactobacillus plantanum* and also enzymes involved in RNA metabolism (Ngai & Ng 2004). The lentin N-terminal sequence revealed similarities with sequences of some endoglucanases near the C-terminal (Ngai &

Ng 2003).

Isolated compounds from mushrooms are promising novel antifungal drugs and further studies are needed to establish *in vivo* antifungal concentrations and determination of reliable doses in living organisms.

4.3 Mushrooms as a Reliable Source of Antioxidants for Disease Prevention

Edible mushrooms have been shown to possess potential as natural antioxidants and there are several reports in the literature. Stojkovic *et al*. (2014a) studied the antioxidant activities of methanolic and ethanolic extracts of *Agaricus bisporus* (J.E. Lange) Imbach and *Agaricus brasiliensis* Wasser, Didukh, Amazonas & Stamets and the latter revealed the highest antioxidant potential. *A. brasiliensis* methanolic and ethanolic extracts also presented the highest total phenolic content (41.72 and 37.93 mg gallic acid equivalent (GAE) per g extract) and revealed the lowest EC_{50} values for the ferricyanide/Prussian blue assay (0.79 mg/mL), DPPH radical scavenging activity assay (1.18 mg/mL), and β-carotene/linoleate assay (0.22 mg/mL). The methanolic extract of *A. bisporus* showed a higher scavenging activity on DPPH radicals (IC 0.139) than hydroxyl (OH^-) radicals (IC 0.149) (Abah & Abah 2010).

Agaricus bohusii Bon is a prized edible mushroom, especially in Serbia and southern Europe where it is very common. Analyzing the results obtained for antioxidant activity, *A. bohusii* revealed a high concentration of total phenolics (89.59 mg GAE/g extract), indicating a high quantity of molecules with reducing capacity (Reis *et al*. 2012a). The EC_{50} values obtained in all the evaluated assays (reducing power, free radical scavenging activity, and lipid peroxidation inhibition) were low (≤ 1.29 mg/mL), indicating a high antioxidant potential of the studied species and correlated to the high concentration of the total phenolics (Reis *et al*. 2012a). The highest “antioxidant power” among 10 *Agaricus* species was noted in *A. silvaticus* (EC_{50} values were the lowest, ranging from

2.08 mg/mL to 5.37 mg/mL, depending on the method), being higher than ascorbic and gallic acids, which are commercial antioxidants (Barros 2008b). Öztürk *et al*. (2011) reported results of three species of *Agaricus* genera and all proved to have antioxidant activity, but none demonstrated better activity than the antioxidant standards. For the β-carotene linoleic acid assay, the methanol extracts of *A. bisporus* (EC_{50} 293.78 µg/mL) showed the highest lipid peroxidation inhibition activity among all the tested extracts, followed by the methanol extract of *A. essettei* (EC_{50} 296.92 µg/mL) and ethyl acetate extract of *A. bitorquis* (EC_{50} 378.48 µg/mL).

Petrovic *et al*. (2014a) reported that the methanolic extract of *Agrocybe aegerita* (V. Brig.) Singer (chestnut mushroom) exhibited high antioxidant activity. The extract gave 17.36 mg GAE/g extract in the Folin–Ciocalteu assay, and revealed high DPPH radical scavenging activity (EC_{50} 7.23 mg/mL). Slightly higher effect was observed in the β-carotene/linoleate assay (EC_{50} 6.11 mg/mL), while ferricyanide/Prussian blue and thiobarbituric acid reactive substances (TBARS) assays showed even higher effects (EC_{50} 2.66 mg/mL and 0.39 mg/mL, respectively). Lo and Cheung (2005) reported antioxidant activity of the methanol crude extract of *A. aegerita* and its fractions, isolated by liquid–liquid partition, using scavenging activity of 2,20-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) radical cation (ABTS) and inhibition of lipid peroxidation of rat brain homogenate. The ethyl acetate (EA) fraction, which showed the most potent antioxidant activity in these two assays, was further fractionated by a Sephadex LH-20 column into four subfractions (EA1–EA4). EA3 exhibited the strongest radical-scavenging activity in the ABTS and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, and showed a similar extent of *in vitro* inhibition of human low-density lipoprotein (LDL) oxidation as caffeic acid. Significant correlation was found between the total phenolic content and the antioxidant activity in the EA fraction and its subfractions.

The antioxidant potential of methanolic extract of *Boletus aereus* Bull. revealed good reducing power (EC_{50} 0.75 mg/mL), scavenging of DPPH radicals (EC_{50} 3.29 mg/mL), inhibition of β-carotene bleaching (EC_{50} 2.89 mg/mL), and lipid peroxidation

inhibition (EC_{50} 0.33 mg/mL) (Stojkovic *et al.* 2013c). Vamanu and Nita (2013) studied the antioxidant potential of ethanolic, methanolic, cold and hot water extracts of *Boletus edulis* Bull. The values for the reducing power of the extracts were in descending order of ethanolic>methanolic>cold water>hot water. For scavenging ability on DPPH radicals, various extracts were effective in the order of ethanolic>hot water>cold water >methanolic extracts; for scavenging ability on ABTS radicals, the order was ethanolic>methanolic>cold water>hot water extracts. For scavenging ability, on nitric oxide and hydroxyl radical activity, the order was ethanolic>methanolic>hot water>cold water extracts. The same trend was also determined for the chelating effect on ferrous ions and for inhibition of lipid peroxidation. The antioxidant activities of three crude polysaccharides (BEPF30, BEPF60, and BEPF80) from *B. edulis* were investigated in *in vitro* systems. Among these three polysaccharides, BEPF60 showed more significant reducing power and chelating activity, and the highest inhibitory effects on superoxide radicals and hydroxyl radicals (Zhang *et al.* 2011). Kosanic *et al.* (2012) studied the acetone extract of the highly valued and endangered species *B. edulis*, originating from Serbia, reporting that it was more effective than α-tocopherol, BHA, and BHT.

Methanolic extract of cultivated and wild samples of *Coprinus comatus* (O.F. Mull.) Gray was tested for its antioxidant potential (reducing power, radical scavenging activity, and lipid peroxidation inhibition) by Stojkovic *et al.* (2013a). Both samples revealed similar reducing power evaluated via the Folin–Ciocalteu assay (24.61–25.98 mg GAE/g extract); however, the cultivated mushroom revealed a higher reducing power, evaluated via the ferricyanide/Prussian blue assay (EC_{50}1.05 mg/mL), but also higher than the reducing power previously reported by Vaz *et al.* (2011) for a Portuguese sample of *C. comatus* (EC_{50} 1.47 mg/mL). The cultivated sample also revealed the highest lipid peroxidation inhibition, since it presented the lowest EC_{50} values for β-carotene/linoleate and TBARS assays (0.36 and 1.15 mg/mL, respectively). β-Carotene bleaching inhibition was also higher than that described for wild *C. comatus* reported by Vaz *et al.* (2011) (EC_{50} 1.26 mg/mL). The wild sample demonstrated the highest

radical scavenging activity (3.76 mg/mL), revealing lower EC_{50} values. Stojkovic *et al*. (2013a) concluded that cultivated samples had the highest antioxidant potential, demonstrating the best results for three of the five assays applied.

Heleno *et al*. (2012a) studied the antioxidant activity of phenolic and polysaccharidic fractions of five mushroom species: *Coprinopsis atramentaria* (Bull.) Redhead, Vilgalys & Moncalvo, *Lactarius bertillonii* (Fr.) Kuntze, *Lactarius vellereus* (Fr.) Fr., *Rhodotus palmatus* (Bull.) Maire, and *Xerocomus chrysenteron* (Bull.) Qul. *C. atramentaria* polysaccharidic and phenolic extracts gave the highest antioxidant activity (lowest EC_{50} values), total phenolics (33.58 mg GAE/g extract), and total polysaccharide content (16.72 mg PE/g extract).

Reis *et al*. (2013) analyzed the antioxidant potential of the methanolic extract of *Cordyceps militaris* (L.: Fr.) Link and revealed low EC_{50} value for lipid peroxidation inhibition (1.05 mg/mL), but high EC_{50} value for DPPH radical scavenging activity (12.17 mg/mL) and also in the Folin–Ciocalteu assay (15.04 mg GAE/g). *C. militaris* exhibited the presence of some antioxidant molecules such as δ-tocopherol or *p*-hydroxybenzoic acid, which may be related to its antioxidant activity (Heleno *et al*. 2010; Reis *et al*. 2012b).

Stojkovic *et al*. (2014b) studied the antioxidant activity of wild and cultivated *Ganoderma lucidum* from Serbia and China, finding that both samples had antioxidant properties. However, *G. lucidum* from Serbia had slightly higher reducing power, DPPH radical scavenging activity, and β-carotene bleaching inhibition (lower EC_{50} values). *G. lucidum* from China gave slightly better results for lipid peroxidation inhibition evaluated by TBARS assay. Mau *et al*. (2002) studied samples of *G. lucidum*, revealing higher reducing power (50% at 0.75 mg/mL), but lower DPPH scavenging activity (50% at 0.5 mg/mL) compared to a sample of *G. lucidum* from Taiwan. However, they gave higher DPPH scavenging activity than samples from Korea (74% at 10 mg/mL) (Kim *et al*. 2008). In general, *G. lucidum* from Portugal, previously studied by Heleno *et al*. (2012b), showed higher antioxidant properties, measured by the same *in vitro* assays.

Laetiporus sulphureus (Bull.) Murrill was studied by Petrovic *et al*. (2014b) to determine *in vitro* antioxidant activities using methanolic and polysaccharidic extracts. In three of the four assays, polysaccharidic extract exhibited the highest activity. Furthermore, for the TBARS assay, the methanolic extract showed the highest activity (EC_{50} 0.78 mg/mL). The observed antioxidant activity may be due to the presence of various antioxidant compounds described in the previous section such as tocopherols (mainly α-tocopherol), organic acids, and phenolic compounds. Other authors (Klaus 2011; Turkoglu *et al*. 2007) reported high antioxidant activity of *L. sulphureus* ethanolic and polysaccharidic extracts. Petrovic *et al*. (2014c) revealed the antioxidant capacity of aqueous, methanolic, and ethanolic extracts of the *L. sulphureus* fruiting body obtained from Serbia and compared different extraction methodologies (classic versus ultrasound assisted). The antioxidant capacity of *L. sulphureus* extracts was determined *in vitro* by measuring the scavenging of free radicals by DPPH or TEAC and the total ferric ion-reducing power and compared with fructose, a well-known monosaccharide. Both the aqueous methanolic and water extracts contained higher total phenolic compounds and showed better antioxidant capacity than the ethanolic extract.

The extraction technique applied had a narrow effect on the antioxidant properties of the mushroom extracts, except for their total phenolic compounds, which increased greatly in the ultrasound-assisted extracts (Turkoglu *et al*. 2007). Organic solvents, such as methanol, ethanol, butanol, dichloromethane, and ethyl acetate, were shown to be effective extractants in numerous species, because most antioxidants are polar components. Thus, the antioxidant activity of dichloromethane and ethyl acetate extracts of the Brazilian commercial strain of *Lentinus edodes* fruiting bodies was significant (Kitzberger *et al*. 2007). Yang *et al*. (2002) and Cheung *et al*. (2003) reported that the most potent compounds with antioxidant activity in *Lentinus edodes* are phenols, with a high positive correlation between phenolic content and DPPH scavenging activity. Methanol and water crude extracts from the shiitake mushroom (*L. edodes*) were investigated for their antioxidant capacity by Cheung *et al*. (2003). The water

extract from *L. edodes* showed more potent radical scavenging activity than methanol – 75.9% (at 20 mg/mL) in the β-carotene bleaching method, 55.4% in the DPPH radical scavenging method (at 6 mg/mL). The antioxidant activities of methanol and water extracts gradually increased with increasing concentration of the extracts. The methanol extract of *L. edodes* showed a strong correlation between its antioxidant activity and total phenolic content. The water extract of *L. edodes* revealed a similar antioxidant activity to the tert-butylhydroquinone (TBHQ) standard (82.2% at 2 mg/mL). It is probable that the antioxidative components in mushroom extracts can reduce the extent of β-carotene destruction by neutralizing the linoleate free radical and other free radicals formed in the system (Cheung *et al*. 2003).

Stojkovic *et al*. (2013c) reported that total tocopherols and total organic acid content observed in methanolic extract of *Morchella esculenta* (L.) Pers. from Serbia gave higher reducing power measured by ferricyanide/Prussian blue assay, and higher DPPH radical scavenging activity than methanolic extract of *M. esculenta* from Portugal. Statistical correlations showed that, among the molecules present in the methanolic extracts, quinic and citric acids were the compounds that contributed more to the DPPH scavenging activity and reducing power measured by ferricyanide/Prussian blue assay. *M. esculenta* from Portugal gave higher radical scavenging activity and reducing power, while the Serbian sample showed higher lipid peroxidation inhibition. Species of the genus *Morchella* originating from Turkey were good β-carotene bleaching inhibitors (63.2% by *M. elatato*, 86.8% by *M. esculenta* var. *umbrina*, at an extract concentration of 0.5 mg/mL) as well as DPPH radical scavengers (40.6% by *M. deliciosato*, 85.4% by *M. conica*, at an extract concentration of 4.5 mg/mL) (Gursoy *et al*. 2009).

The ethanolic extracts of *Pleurotus pulmonarius*, *P. ostreatus*, *P. djamor* var. *djamor*, and *P. djamor* var. *roseus* were screened for their antioxidant activity by Arbaayah and Umi Kalsom (2013). Inhibition concentration at 50% (IC_{50}) for each extract to scavenge DPPH radicals was detected from 2.75 mg/mL to 12 mg/mL, where *P. djamor* var. *djamor* showed the lowest

IC_{50} value among all tested mushrooms. Thus, the greatest ability to reduce ferricyanide complex to ferrous form was observed in *P. djamor* var. *djamor* at a concentration of 10 mg/mL in both first (1.23) and second flushes (1.23). Meanwhile, the highest total phenols were found in *P. djamor* var. *djamor* extract (51.94 mg TAE/g dry weight of extract). A study by Iwalokun *et al*. (2007) revealed the antioxidant activity of petroleum ether (PE) and acetone (AE) extracts of *P. ostreatus* fruiting body. Antioxidant activity of the extracts using DPPH and ABTS methods revealed disparate vitamin C equivalent antioxidant capacity (VCEACs) of 3.6–3.8 mM for PE and 4.1–4.4 mM for AE, which are comparable to those in green tea infusion (6.2–6.4 mM). Akata *et al*. (2012) studied *P. ostreatus*, revealing a potent free radical scavenging activity (96.16 %) at 2.72 mg/mL of extract concentration.

Studies on the *in vitro* antioxidant potential of the methanolic and ethanolic extract of *Phellinus linteus* as well as selected fractions (polysaccharides, glucans, and triterpenoids) were performed by Reis *et al*. (2014a). It was concluded that the methanolic extract of *P. linteus* revealed the lowest EC_{50} values for DPPH radical scavenging activity (70 μg/mL), reducing power (50.5 μg/mL), and lipid peroxidation inhibition, for β-carotene bleaching inhibition (114 μg/mL) and TBARS inhibition (8 μg/mL). Among the assayed fractions, glucans showed the lowest antioxidant activity. Highest activity among assays was obtained for TBARS formation inhibition, while the worst values resulted from β-carotene bleaching inhibition. Nevertheless, *P. linteus* proved to have high potential for antioxidant purposes, since the obtained EC_{50} values were lower than those resulting from other wild edible species, which varied from 20.02 to 0.68 mg/mL (Pereira *et al*. 2012). In a study by Song *et al*. (2003), *P. linteus* was shown to scavenge the DPPH radical directly over a concentration range of 10 μg/mL (30% inhibition) to 300 μg/mL (85% inhibition), suggesting that the stable free radical scavenging activity of *P. linteus* is comparable to that of vitamin C.

Reis *et al*. (2014b) studied the antioxidant properties of methanolic extracts of the wild mushroom *Suillus granulatus* (L.) Roussel from Serbia and Portugal. The Serbian sample showed the highest reducing power, with the highest content in

total phenolics assessed through the Folin–Ciocalteu assay (44.36 mg/GAE g extract) and the lowest EC_{50} value for the ferricyanide/ Prussian blue assay (0.41 mg/mL). It also revealed the highest radical scavenging activity, evaluated with the DPPH radical scavenging activity assay (0.89 mg/mL), and lipid peroxidation inhibition assessed via the TBARS assay (0.02 mg/mL). The exception was verified with the evaluation of the lipid peroxidation inhibition measured through the β-carotene/linoleate assay, where both samples presented similar EC_{50} values with no significant differences between them (0.45 and 0.48–mg/mL). Ribeiro *et al.* (2006) also presented antioxidant activity results for *S. granulatus*, revealing a moderate antioxidant potential (evaluated via the DDPH radical scavenging activity).

The methanolic extract of *Tirmania pinoyi* (Maire) Malencon showed *in vitro* antioxidant activities evaluated by four different assays, presenting moderate reducing power (EC_{50} 1.80 mg/mL), scavenging of DPPH radicals (EC_{50} 6.41 mg/mL), inhibition of β-carotene bleaching (EC_{50} 28.38 mg/mL), and lipid peroxidation inhibition using the TBARS assay (EC_{50} 2.24 mg/mL). The antioxidant activity reported is lower than that demonstrated by trolox (standard). However, comparison of extracts with pure compounds should be avoided, because they are individual/ purified compounds and not mixtures (in the crude extract the concentration of each individual compound is certainly much lower) (Stojkovic *et al.* 2013b).

4.4 Could Mushrooms Be Used as Cytotoxic and Antitumor Agents?

As mentioned above, mushrooms are important dietary components in some cultures, some of them being traditionally used for the treatment of various conditions, including cancer (Xu *et al.* 2012). Identification of active principles in extracts, i.e. isolation of new antitumor substances from mushrooms, has became a matter of great importance, giventhe complexity and

distribution of various cancer types in the population worldwide (Zong *et al*. 2012). A great variety of compounds and complex fractions have been isolated and/or purified from medicinal as well as some edible mushrooms, with special emphasis on anticancer and cancer preventive activity (Xu *et al*. 2012). Amongst the broad spectrum of constituents in medicinal and edible mushrooms, these activities are mainly attributed to polysaccharides, various polysaccharide-protein/peptide complexes, lectins, terpenoids, sterols, etc. Special interest is devoted to polysaccharides from the fungal cell walls because of their immunomodulatory activity, being biological response modifiers (BRM) that prevent carcinogenesis, but they also show direct anticancer effects and prevent tumor metastasis (Popovic *et al*. 2013).

4.4.1 Cytotoxic Features of Wild Mushroom Extracts

Mushroom extracts are increasingly consumed as dietary supplements because of their properties, including the enhancement of immune function and antitumor activity (Finimundy *et al*. 2013). It is well established that mushroom extracts contain a wide variety of compounds such as polysaccharides, protein, fiber, lectins, and polyphenols, each of which may have pharmacological effects. More than 30 species of medicinal mushrooms are currently identified as sources of biologically active metabolites with potential anticancer properties (de Silva *et al*. 2012). The properties and mechanisms of mushroom extracts that have been evaluated are outlined in Table 4.2.

Table 4.2 Antitumor activity of mushroom extracts.

Mushroom species	Active compounds/ extracts/ fractions	Cell type	Activity/ results	Mechanism of action	Reference
Amauroderma rude	Hot water extract	Human breast	ns	Induction of	(Jiao *et al*. 2013)

(Berk.) Torrend 1920		cancer cell lines MT-1, MDA-MB231, 4 T1, MDA-MB468, MCF7		apoptosis	
Antrodia camphorata (Zang & Su) Wu, Ryvander, Chang 1997	Cold water extract	Human breast cancer cell lines MDA-MB-453 and BT-474	IC_{50} values 220 and 240 µg/mL for MDA-MB-453 and BT-474 cells respectively	Inhibition of cell growth and induction of apoptosis through the induction of ROS, depletion of HER-2/neu, and disruption of the PI3K/Akt signaling pathway	(Lee *et al*. 2012)
	Fermentation culture	Human ovarian carcinoma (SKOV-3) cells	At 240 µg/mL colony formation was reduced by over 90%	Modulation of HER-2/neu signaling pathway	(Yang *et al*. 2013)

		compared to the untreated control cells			
Antrodia cinnamomea Cnag & Chou 1995	Ethanolic extract	Murine leukemia WEHI-3 cells	ns	Inhibition of the proliferation and migration of WEHI-3 cells, MMP-9 protein expression reduction	(Liu *et al.* 2013)
Clitocybe alexandri Gillet 1884	Ethanolic extract	Human nonsmall cell lung cancer NCI-H460	ns	S-phase cell cycle arrest	(Vaz *et al.* 2012a)
Ganoderma lucidum	Commercially available extract reishimax glptm	Mice injected with IBC cells	Reduction of tumor growth and weight by 45%	Reduction in expression at both the gene and protein level of important molecules in the PI3K/Akt/mTOR and	(Suarez-Arrayo *et al.* 2013)

				MAPK signaling pathways	
Hericium erinaceus (Bull.) Pers 1797	50% Ethanol extract	CT-26 mouse colon carcinoma cell	42% inhibition at 1 mg/mL	Suppression of ERK and JNK activation, inhibition of lung metastasis *in vivo*	(Kim *et al*. 2013)
Lentinula edodes	Aqueous extract	Human tumor cell lines laryngeal carcinoma (Hep-2), cervical adenocarcinoma (HeLa)	IC_{50} values from 0.46–1.03 µg/mL	Apoptosis induction	(Finimundy *et al*. 2013)
	Ethanol extract	HepG2 human hepatocellular carcinoma	ns	Apoptosis induction through caspase-3 and -8 death receptor pathway	(Yukawa *et al*. 2012)
Lignosus rhinocerus (Cooke) Ryvander 1972	Cold water extract	Human breast carcinoma MCF-7	IC_{50} 96.7 µg/mL	—	(Lee *et al*. 2012)
		Human lung carcinoma A549	IC_{50} 466.7 µg/mL		

Pleurotus pulmonarius (Fr.) Quel 1872	Hot water extract	Human liver cancer cell lines Huh7, Hep 3B, SMMC-7721 and HepG2	ns	Inhibition of VEGF-mediated autocrine regulation of PI3K/AKT	(Xu *et al.* 2012)
Pleurotus sajor-caju	Aqueous extract	Human tumor cell lines laryngeal carcinoma (Hep-2) and cervical adenocarcinoma (HeLa)	IC_{50} 0.25 0.78 µg/mL	Apoptosis induction	(Finimundy *et al.* 2013)
Ramaria flava (Schaeff.) Quel. 1888	Ethanol extract	BGC-803, NCI-H520, MDA-MB-231	IC_{50} ranged from 66.54 to 743.99 µg/mL for the MDA-MB-231 and BGC-803 cell lines respectively	ns	(Liu *et al.* 2013)
Suillus collinitus (Fr.) Kunteze 1898	Methanolic extract	MCF-7 human breast cancer cell line	ns	Increases p53 expression and causes	(Vaz *et al.* 2012b)

				apoptosis	
Suillus luteus (L.) Roussel 1796	Methanolic extract	MCF-7 human breast cancer cell line, NCI-H460 human nonsmall cell lung cancer, AGS human gastric cancer, HCT-15 human colon cancer	GI_{50} values ranged from 17.75 to 32.25 µg/mL for the HCT-15 and MCF-7 cells, respectively	Increases p53 expression and causes apoptosis	(Dos Santos *et al*. 2013)
Tricholoma giganteum Massee 1912	80% Ethanol extract	Ehrlich ascites carcinoma	ns	Apoptosis induction	(Chatterjee *et al*. 2013)

ns, nonspecified; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor.

The reported results are mainly from *in vitro* studies and as a hint of their potential therapeutic value, they mark the very first steps in preclinical screening. Often they are also used as advertising arguments for traditional medicines (Finimundy *et al*. 2013).

4.4.2 Mushroom Polysaccharides, β-, and α-Glucans as Antitumor Agents

Polysaccharides are biopolymers, consisting of monosaccharide units linked through glucoside bonds with high ability to carry

biological information due to numerous structural variations. Many of them have been previously mentioned as good antimicrobial and/or antifungal compounds. Moreover, they have been shown to exert *in vitro* antiproliferative/cytotoxic and antitumor activity in animal models (Zong *et al*. 2012). Polysaccharides are mainly used as an adjuvant therapy in cancer treatment (Liang *et al*. 2011). Several structural features are known to affect these biological activities, primarily specific structural features, molecular weight, backbone linkage, degree of branching, and side-chain units, as well as monosaccharide composition (Lo *et al*. 2011).

Mushroom polysaccharides that exert antitumor activities have been isolated from fruiting bodies, cultured mycelia, and culture filtrates of basidiomycetes (Ren *et al*. 2012). Considering backbone structure, it is known that glucose residues linked by β-(1→3)-glycosidic bonds with attached β-(1→6) branch points exhibit strong antitumor and immunostimulating properties (Ren *et al*. 2012). In the following overview, besides well-known and commercially available products from a polysaccharide source, such as schizophyllan, lentinan, and grifolin, a brief report on other polysaccharides that are being currently investigated for their potential use in mycotherapy of cancer will be given.

Low molecular weight polysaccharide (LMW-ABP) isolated from the fruiting bodies of *Agaricus blazei* (syn. *A. brasiliensis*) inhibited tumor growth and angiogenesis *in vivo* by downregulating vascular endothelial growth factor (VEGF). It was further shown that this polysaccharide inhibited tumor cell adhesion via depressing E-selectin protein expression and also NF-κB protein expression, so it may be a promising therapeutic agent against E-selectin-mediated neoplasm metastasis (Yue *et al*. 2012). From the fruiting bodies of the same species, a heteropolysaccharide (MW 4.2×10^5 Da), consisting of glucose, mannose, and galactose in a molar ratio 1:1:1, was purified, and cytotoxicity was tested in osteosarcoma human osteoblast cells as well as in normal human osteoblast cells (Wu *et al*. 2012a). A heteroglucan polysaccharide isolated from *Astraeus hygrometricus* (Pers.) Morgan induced tumor regression in Dalton lymphoma-bearing mice, and a possible mechanism, the

elevation of macrophage and NK cells activation, with increase in Th1 cytokine production (Mallick *et al*. 2010), was suggested. Apart from β-glucans, a water-soluble heteropolysaccharide consisting of galactose, mannose, fucose, and glucose in a molar ratio of 1.24:1:0.95:0.88 was purified from the medicinal maitake mushroom (*Grifola frondosa* (Dicks.) Gray). This polysaccharide inhibited colon 26 tumor growth in BALB/cA mice, to a level achieved by the reference β-glucan, and the effect is thought to be associated with induced cell-mediated immunity (Masuda *et al*. 2009). An alkaline-soluble polysaccharide (MW 6.3 kDa) isolated and purified from *Inonotus obliquus* consisted of rhamnose, xylose, manose, galactose, glucose, and galacturonic acid in a molar ratio of 3.09:1.61:2.06:4.45:19.7:1, and showed excellent activity against solid tumor sarcoma 180 formation in mice; the activity was associated with a potent immunostimulating effect of this polysaccharide (Zhang *et al*. 2011). Another heteropolysaccharide (MW 93 kDa) was extracted and purified from *I. obliquus*, but was water soluble and consisted of rhamnose, mannose, and glucose in molar ratios of 1.0:2.3:1.7. For this polysaccharide, no significant *in vitro* cytotoxic effect was observed, but it exerted a significant antitumor effect in human gastric carcinoma SGC-7901-bearing nude mice. Similar to other polysaccharides, the authors suggested possible mechanisms related to cancer prevention, immune enhancement, and direct tumor inhibition (Fan *et al*. 2012). One of the polysaccharide fractions isolated from fruiting bodies of *Tricholoma matsutake* (S. Ito & S. Imai) Singer, unlike other purified fractions of this mushroom, was found to consist of glucose, galactose, and mannose with a molar ratio 5.9:1.1:1.0. This fraction exerted strong antiproliferative activity on HepG2 and A549 cell lines in an MTT test (You *et al*. 2013).

Several investigations revealed that water solubility of heteropolysaccharides could be one of the key features for increased antitumor activity (Ren *et al*. 2012; Rop *et al*. 2009). Apart from carboxymethylation, it has been shown that O-sulfonated derivatives of native water-insoluble (1→3)-α-D-glucans, isolated from fruiting bodies of *Lentinus edodes*, exert inhibition of growth of solid tumor sarcoma 180 implanted in

mice. Also, cytotoxic activity of O-sulfonated glucans exerted cytotoxic activity in an MTT assay on the same cell line. O-Sulfonation increased antitumor and cytotoxic activities of naturally occurring glucans, in both *in vitro* and *in vivo* tests (Unursaikhan *et al*. 2006). β-Glucans are fundamental building blocks in fungi, since their cell walls are composed of two polymers, chitin and β-glucan, that are interlinked by covalent bonds and hydrogen bridges, which create a strong foundation for chitin fiber networks incorporated in the glucan matrix. β-Glucans are polysaccharides where glucose is a sole monomer unit, from tens to thousands of kilodaltons, more or less soluble in water, which increases with temperature of the solvent. Glucans that are isolated from mushrooms are mainly β-1,3-D-glucan or β-1,6-D-glucan (Rop *et al*. 2009). Even though the chemical structure of β-glucans in the cell walls of fungi has not been examined fully, it is known that immunomodulating activity is mainly dependent on a single helix glucan structure which can interact with and/or link to immunoglobulins present in blood serum. Several structural features contribute to these effects such as higher degree of substitution, presence of hydrophilic groups on the helix surface, and higher molecular weight.

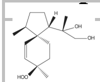
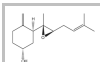
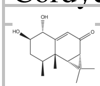
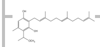
In the human body, glucans are intensively oxidized and the metabolites formed are temporary and less effective than β-glucans themselves (Rop *et al*. 2009). Basically, the underlying mechanisms for antitumor activities of β-glucans such as lentinan, schizophyllan, and grifolin include stimulation of hematopoietic stem cells, activation of the alternative complement pathway, and activation of immune cells such as lymphocytes, macrophages, dendritic cells (DC), natural killer (NK) cells, Th cells, Tc cells, and B cells (Wiater *et al*. 2011). Apart from β-glucans, immunostimulatory and potential anticancer activity of α-glucans was also shown. A branched α-(1→4)-glucan (L10) purified from *Lentinula edodes* induced a significant reduction of viability (66% to 37%) of irradiated human lung adenocarcinoma A549 cells co-cultured with monocytes (THP-1) by Toll-like receptor 4-mediated induction of THP-1 (Lo *et al*. 2011). The authors stated that L10 monocytes have the potential to enhance the antitumor immune response and antitumor effect of radiotherapy. A low molecular complex of glucans (20 kDa) derived from *Agaricus*

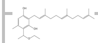
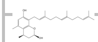
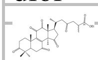
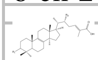
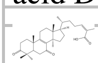
blazei consisted of α-(1→4)-glucan and β-(1→6)-glucan, and demonstrated *in vitro* selective cytotoxicity in MethA tumor cells, without affecting normal cells (Fujimiya *et al.* 1999).

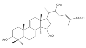
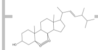
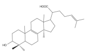
4.4.3 Cytotoxic Potency of Terpenoids and Related Compounds from Mushrooms

Certain classes of terpenoid compounds were isolated from some mushroom species and their structure was completely elucidated. The most important class is lanostane triterpenes, isolated from species such as *Ganoderma lucidum*, *Poria cocos*, *Laetiporus sulphureus*, *Inonotus obliquus*, and *Anthrodia camphorata*, that were investigated for their cytotoxic or apoptotic effects (Rios *et al.* 2012). In the following section, a brief overview of terpenoid compounds is given, and some of the structures are provided in Table 4.3.

Table 4.3 Some of the compounds isolated from medicinal mushrooms that exert cytotoxic or apoptotic effects.

Compound	Exerted activity	Reference
 Cordycepol C	Cytotoxic effects in HeLa, A549, HepG2 and MCF 7 cell lines	(Sun *et al.* 2013)
 Cordycol		
 Nambinone C	Cytotoxic effect in NCI/H187 cells	(Kanokmedhakul *et al.* 2012)
 4-(1-methoxyethyl)-5-methyl-2-[(2E,6E)-3,7,11-trimethyldodec-2,6,10-	Cytotoxic effect in human lung carcinoma and human and mouse melanoma cell lines	(Song *et al.* 2009)

trienyl]benzene-1,3-diol		
 4-(1-ethoxyethyl)-5-methyl-2-[(2E,6E)-3,7,11-trimethyldodec-2,6,10-trienyl]benzene-1,3-diol	Cytotoxic effect in human lung carcinoma and human and mouse melanoma cell lines	(Song *et al*. 2009)
 (2R*, 4R*)-3,4-dihydro-4,5-dimethyl-8-[(2E,6E)-3,7,11-trimethyldodec-2,6,10-trienyl]-2H-[1]benzopyran-2,7-diol	Cytotoxic effect in human lung carcinoma and human and mouse melanoma cell lines	(Song *et al*. 2009)
 Ethyl 3,7,11,12,15,23-hexaoxo-5α-lanost-8-en-26-oate	Cytotoxic effect in B16F1, B16F10, Huh-7, MCF 7 and A 2058	(Huang *et al*. 2012)
 $R_1 = O$; $R_2 = OAc$ Astraodoric acid A $R_1 = O$; $R_2 = OH$ Astraodoric acid B $R_1 = \alpha$-OH; $R_2 = OAc$ Astraodoric acid D	Cytotoxic effect in KB, NCI-H187 and MCF 7 cell line	(Arpha *et al*. 2012)
 Ganoderic acid DM	Antiprostate cancer activity and cytotoxic effect in MCF 7 cell line	(Wu *et al*. 2012b)

 Ganoderic acid T	Three human carcinoma cell lines	(Liu *et al*. 2012)
 Ergosterol peroxide	Induction of cell death of miR-378-transfected cells Cytotoxic effects in human breast and prostate cell lines	(Wu *et al*. 2012c) (Ma *et al*. 2013)
 Trametenolic acid	Cytotoxic effects in human breast and prostate cell lines	(Ma *et al*. 2013)

4.4.4 Mushroom Sterols Inhibit the Growth of Carcinoma Cell Lines

Various ergosterol derivatives have been isolated from mushrooms such as *Lentinus edodes*, *Polyporus umbellatus*, and *Agaricus blazei*, mainly from the lipid fraction (Takaku *et al*. 2001). Oral administration of ergosterol (400 and 800 mg/kg for 20 days) to sarcoma 180-bearing mice significantly reduced tumor growth without side-effects, such as decreases in body weight, epididymal adipose tissue, thymus, spleen weight, and leukocyte numbers. Ergosterol did not induce cytotoxic effects in tumor cells but acted as an antiangiogenic substance in two *in vivo* models of tumor- and Matrigel-induced neurovascularization (Takaku *et al*. 2001). Ergosterol peroxide induced death of the miR-378-transfected cells; miR-378 are expressed in a number of cancer cell lines. These data indicate that ergosterol peroxide may be a new reagent for overcoming the problem of drug resistance in tumor cells (Wu *et al*. 2012c). Ergosterol peroxide and trametenolic acid (see Table 4.3) isolated from *Inonotus obliquus* exerted cytotoxic activity in human prostate and breast carcinoma cell lines (Ma *et al*. 2013).

4.5 Controlling Obesity, Metabolic Syndrome, and Diabetes Mellitus with

Mushrooms

Central obesity is one of the components of the metabolic syndrome (MS). MS is a group of conditions that occur together, including increased blood pressure, hyperglycemia, excess body fat around the waist, and abnormal cholesterol levels. Taken together, these conditions increase the risk for heart disease, stroke, and diabetes. Having MS means having three or more disorders related to the metabolism at the same time (Torpy *et al.* 2006).

Many studies have shown positive effects of diet and dietary constituents in prevention and treatment of MS which could be connected with low glycemic properties and dietary fiber content (Torpy *et al.* 2006). The β-linkage in β-glucans makes them indigestible but highly fermentable in the cecum and colon. Also, β-glucans possess the ability to form highly viscous solutions in the human gut, which could be connected with their effects in the metabolic syndrome. These effects include lowering postprandial glucose and insulin responses, decreasing cholesterol levels and potentiating the feeling of satiety, delayed gastric emptying with increased viscosity causing slow digestion and absorption and therefore decreasing glucose transport to enterocytes (El Khoury *et al.* 2012). Experimental data on the effect of β-glucans in obesity were contradictory; most authors reported that fiber intake reduces the level of weight gain but others demonstrated opposite results. An attempt was made to explain this inconsistency through the following factors: differences in the β-glucan dose, the molecular size of β-glucan, the composition of food, the process of food preparation, etc. However, no single factor can adequately explain the inconsistency (Soo *et al.* 2006). However, positive effects were more probably connected with reduced hunger sensation (Dikeman *et al.* 2006).

Several *in vivo* studies have shown positive effects of mushrooms in weight control although body weight was not the primary concern of these studies. Dietary supplementation in females with the combination of *A. blazei* and *L. edodes* has shown body weight reduction (Kweon *et al.* 2002). Dried powder of *Auricularia auricula-judae* (Fr.) Quel., suspension of *Coprinus*

comatus, α-glucan of *Grifola frondosa*, and ethanol extract of *Pleurotus ostreatus* inhibited body weight increase in healthy and diabetic patients. *Lentinus edodes* and rice with *L. edodes* mycelium in doses of 300 μg/mL had positive effects on obesity, with lipid accumulation decreasing by 78% and 74%, respectively (Kim *et al*. 2013).

The β-glucan-rich extract from *Pleurotus sajor-caju* has been shown to prevent obesity and could be useful as adjuvant therapy. According to Kanagasabapathy *et al*. (2013), this extract induced the expression of hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL), while downregulating the expression of peroxisome proliferator-activated receptor γ (PPAR-γ), sterol regulatory binding protein-1c (SREBP-1c), and lipoprotein lipase (LPL). PPAR-γ is expressed selectively in adipose tissues and promotes the differentiation and proliferation of preadipocytes, thereby causing an increase in fat mass, while SREBP-1c is considered as a key regulator for fatty acid and triglyceride synthesis. LPL is a key enzyme that regulates lipid disposal in the body, and controls the hydrolysis of circulating triglycerides in the lipoprotein particles in order to facilitate the uptake of fatty acids into the cells (Kanagasabapathy *et al*. 2013). Supplementation with β-glucan-rich extract reduced adipose tissue differentiation and increased lipolysis in adipocytes. These new and interesting findings could be one of the explanations of the reduction of body weight in the high-fat diet *in vivo* studies (Kanagasabapathy *et al*. 2013).

In vitro experiments involved inhibition of α-amylase and α-glucosidase that hydrolyze α-bonds of large, α-linked polysaccharides. The most complete study, perfomed in 2010, screened hot water and ethanol extracts of 195 species of wild and cultivated mushrooms (Ohuchi *et al*. 2010). When they were compared using IC_{50} data, α-glucosidase inhibition activity of these mushrooms was stronger than α-amylase inhibition activity, which was correlated with nojirimycin derivative contents. Active nojirimycin derivatives were identified as α-homonojirimycin and 7-O-β-D-glucopyranosyl-α-homonojirimycin, in four mushrooms such as *Boletus pseudocalopus* Hongo, *Cortinarius armillatus* (Fr.) Fr., *C. alboviolaceus* (Pers.) Fr., and *Dictyophora*

indusiata (Vent.) Desv. (Ohuchi *et al*. 2010).

A water-soluble polysaccharide was isolated from *Inonotus obliquus* and exhibited inhibitory activity against α-glucosidase with IC_{50} values of 93.3 μg/mL, whereas it had no effective inhibition on α-amylase (Chen *et al*. 2010). Moreover, inotodiol and trametenolic acid from ethyl acetate extract of *I. obliquus* were found to have an inhibitory effect on α-amylase activity (Lu *et al*. 2010).

Additionally, ethanol extract from *Tremella fuciformis* Berk. significantly inhibited α-glucosidase from small intestine of pig and rat (about 42% and 35%, respectively), and stimulated glucose uptake in 3 T3-L1 mature adipocytes (about 100%); this activity was higher than that of maitake (*Grifola frondosa*), a well-known antidiabetic mushroom. The major components were 1-monooleoylglycerol and 1-monopalmitoylglycerol (Jeong *et al*. 2008).

The *in vivo* experiments on antidiabetic and hypoglycemic activities are mostly done on rats and mice with insulin-dependent diabetes mellitus induced by streptozotocin and alloxane, as well on genetically diabetic mice with non-insulin-dependent diabetes mellitus. The mechanisms of action are still unknown, but most of these studies were performed using medicinal mushrooms containing β-glucans. This polysaccharide could restore pancreatic tissue function, causing an increase in insulin output by functional β-cells, which results in lowering glucose level in the blood (Misra *et al*. 2009; Qiang *et al*. 2009; Xiao *et al*. 2011). One of the most promising mushrooms was *G. frondosa*, which could control all signs of MS: excess body weight, cholesterol, diabetes, and hypertension (Donatini *et al*. 2011). The results of Hong *et al*. (2007) suggested that α-glucan from *G. frondosa* exhibited an antidiabetic effect on KK-Ay mice, which might be related to its effect on insulin receptors (i.e. increasing insulin sensitivity and improving the insulin resistance of peripheral target tissues).

In contrast, a report by Kim *et al*. (2010) showed that polysaccharide PLP isolated from *Phellinus linteus* inhibited the expression of inflammatory cytokines, including interferon (IFN)-γ, interleukin (IL)-2, and tumour necrosis factor (TNF)-α by T-

helper 1 (Th1) cells and macrophages, but upregulated IL-4 expression by Th2 cells in nonobese diabetic mice. Polysaccharides from *P. linteus* did not prevent streptozotocin-induced diabetic development in mice, but could inhibit the development of autoimmune diabetes in nonobese diabetic mice by controlling cytokine production (Kim *et al*. 2010). Water-soluble extract of *Panellus serotinus* significantly decreased the serum level of monocyte chemoattractant protein-1 (MCP-1), which is known to exacerbate insulin resistance, and at the same time, the serum level of adiponectin, which plays a protective role against the MS, was significantly increased by the ethanol extract of the same mushroom (Inafuku *et al*. 2012). Similar results were obtained with *Sparassis crispa* Desjardin & Zheng Wang; when applied to the diet of type 2 diabetic mice, it regulated the levels of adiponectin, glucose, and insulin in blood serum (Yamamoto & Kimura 2010). The importance of adiponectin is well known, because it plays an important role in regulating glucose levels as well as fatty acid metabolism. Therefore, a low level of adiponectin is an independent risk factor for developing MS (Díez *et al*. 2003; Renaldi *et al*. 2009).

In the literature, there have been interesting results in the regulation of glycemia using mushrooms and antidiabetics drugs, demonstrating synergistic effects. Aqueous extract of *Pleurotus pulmonarius* exhibited synergistic effects with the antidiabetic drug glyburide in alloxane-induced diabetic mice, according to Badole *et al*. (2008). Supplementation with mushrooms or their extracts could be one of the dietetic measures in controlling sugar level in diabetics or persons with MS (Badole *et al*. 2008). The active compounds were in most cases polysaccharides, but also lectins and nonlectin compounds (*Agaricus campestri*s Scop., *A. bisporus*), guanidine and dehydrotrametenolic acid (*Laricifomes officinalis* (Vill.) Kotl. & Pouzar, *Laetiporus sulphureus*, *Wolfiporia cocos* (F.A. Wolf) Ryvarden & Gilb.) (Ahmad *et al*. 1984; Gray & Flatt 1998; Rathee *et al*. 2012; Sato *et al*. 2002). Insulin release in isolated Langerhans rat islets was mostly enhanced by lectins, but also nonlectin compounds, which possessed insulin-like activity (Ahmad *et al*. 1984; Gray & Flatt 1998).

These are promising findings, but the chemistry and pharmacology of peptides need further studies. A large number of animal studies have been conducted, but clinical studies in humans are very rare and involve a small number of patients. In 2007, Khatun *et al.* performed a randomized, double-blinded and placebo-controlled clinical trial with 72 patients which showed beneficial effects of supplementation with *Agaricus blazei* in combination with metformin and gliclazide. The duration of this study was 24 days, with a specific designed protocol: supplementation with mushroom for seven days, seven days without supplementation and then another seven days with supplementation. Measurements were done at the beginning of the study and every seven days. Supplementation with oyster mushroom significantly reduced systolic and diastolic blood pressure and lowered plasma glucose, total cholesterol, and triglycerides significantly, without significant change in weight and high-density lipoprotein (HDL) cholesterol. When the mushrooms were withdrawn, there were significant increases of diastolic blood pressure, fasting plasma glucose, two-hour postchallenge glycemia, and total cholesterol and triglycerides; no significant change was observed in weight, systolic blood pressure, and HDL cholesterol and no adverse effects on liver and kidney (Khatun *et al.* 2007).

Hsu *et al.* (2007) also performed a clinical randomized, double-blind, placebo-controlled trial with 72 patients with type 2 diabetes. These patients were supplemented with *Agaricus blazei* extract in doses of 1500 mg daily for 12 weeks in combination with gliclazide and metformin. The authors suggested that *A. blazei* extract improved insulin resistance among these patients by increasing the adiponectin concentration.

4.6 Conclusion

Mycotherapy is a novel discipline describing the beneficial health effects of medicinal and edible mushrooms. Most of the studies available in the literature have focused on screening the antibacterial and antifungal activity of mushroom extracts, rather than of isolated compounds. After elucidation of their mechanism

of action, LMW or HMW mushroom compounds could be used to develop antibiotics for pathogenic or contaminant microorganisms.

Numerous studies on the antioxidant activity of wild and cultivated mushrooms have shown that research on the biological effects of mushrooms can contribute to human health and quality of life. The assumption that there are thousands more species waiting to be discovered with potential benefits for humans will encourage future research in this area.

Treating cancer with mushrooms is a promising avenue in the current scientific and medical battle against serious disease. Studies to date have identified a number of compounds and elucidated underlying mechanisms. Further research studies focused on cancer treatment with mushrooms, especially clinical trials, are needed to validate the usefulness of mushrooms and their compounds, either alone or in combination with existing therapies.

The beneficial effects of mushrooms in moderating metabolic syndrome symptoms were known from both traditional and conventional medicine. Recent published data suggest very potent hypoglycemic, cholesterol, and triglyceride lowering activity from supplementation with edible mushrooms or their extracts, the main target being to improve insulin resistance. Also, several studies have shown positive results in body weight reduction, suggesting possible influence in adipose tissue differentiation and increased lipolysis in adipocytes. The mechanisms of action are still to be revealed but will probably point to β-glucans and lectins as the most important active compounds, but also smaller compounds such as triterpenes and phenolic compounds.

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5
The Use of Mushrooms in the Development of Functional Foods, Drugs, and Nutraceuticals

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5.1 Introduction

It is so determined by Nature that right from the beginning everyone's life, at least for a certain period of time, depends on the strict fulfillment of Hippocrates' advice: "Let food be thy medicine and medicine be thy food" (Milner 2002). In this context, the use of mushrooms to improve health represents an important cultural heritage as they have been used since time immemorial as a source of highly tasty/nutritional foods and medicinal preparations according to traditional ecological knowledge transmitted through the generations by the greatest early civilizations (Pereira *et al*. 2012; Stamets 2002; Wasser 2010a; Wasser & Weis 1999). Sometimes the health benefits of their use were so impressive that ancient people converted the result observed into long-lived stories of mushroom magic (Hobbs 2000). Thus, experiences of ethnomycological uses of mushrooms deserve a modern evaluation.

Although for most people mushrooms are still considered as one of

the curiosities of Nature, by combining tradition and new information, edible and medicinal mushrooms are now attracting more attention. Looking at the health-related issues of the new millennium, the driving forces for this upsurge of interest in mushrooms include aging, projections of the global burden of cancer and chronic noncommunicable diseases (e.g. cardiovascular diseases, diabetes, obesity, and neurodegenerative disorders, among others), with cancer being the main cause of death around the world in the last few years, and pandemic diseases like acquired immune deficiency syndrome (AIDS). A cost-analysis carried out at Harvard University suggested that if current health trends are not addressed, the costs to medical services associated to chronic nontransmissible diseases will rise to US$47 trillion in the next 20 years (Bloom *et al*. 2011). In consequence, there is an increase in consumers' interest in modifying lifestyles, particularly through a health-promoting and/or disease-preventing diet (Chang & Wasser 2012; Keservani *et al*. 2010; Mahabir & Pathak 2013; Shahidi 2012).

Mushrooms are emerging as a vital component of the human diet and several comprehensive reviews of their nutritional value have been presented (Chang & Buswell 2003; Kalač 2013; Khan & Tania 2012; Ulziijargal & Mau 2011) (see also Chapter 3). Thus, mushrooms have become attractive as a functional food and as a source of drugs and nutraceuticals (Chang 2009; Ferreira *et al*. 2009; Patel *et al*. 2012) and world production in 2012 was 30 million tons (Wasser 2014). Mushrooms as functional food and nutraceuticals (dietary supplements) can help in the intervention of subhealth states and may prevent the full-blown consequences of life-threatening diseases (Vikineswary & Chang 2013).

Several mushroom species are known to possess medicinal value and some are already being used for such purposes. Of the known mushroom species, approximately 700 are considered to be safe with medicinal properties (Wasser 2010a). Pharmacological effects have been demonstrated for many traditionally used mushrooms, including species from genera *Ganoderma*, *Lentinus (Lentinula)*, *Agaricus*, *Auricularia, Flammulina*, *Grifola*, *Hericium*, *Pleurotus*, *Trametes (Coriolus)*, *Schizophyllum*, *Lactarius*, *Phellinus*, *Cordyceps*, *Inonotus*, *Inocybe*,

Tremella, and *Russula* (Lindequist *et al*. 2005; Patel & Goyal 2012; Stamets 2002; Vikineswary & Chang 2013). In this wonderful world, *Ganoderma*, mushroom of immortality, has been considered as king of medicinal mushrooms, followed by *Lentinula* and others, including *Pleurotus* (Patel *et al*. 2012).

Fruiting bodies as well as mushroom mycelia have a broad range of bioactive properties (see Chapter 4). Mushrooms are thought to exert approximately 130 pharmacological functions such as antitumor, immunomodulatory, antigenotoxic, antioxidant, antiinflammatory, hypocholesterolemic, antihypertensive, antiplatelet-aggregating, antihyperglycemic, antimicrobial, and antiviral activities (Lindequist 2013; Patel *et al*. 2012; Paterson & Lima 2014). Many controlled studies have investigated this long list of medicinal actions, thus upgrading mushrooms to today's world of evidence-based medicine (Wasser 2014).

Mushrooms are natural bioreactors for the production of compounds with human interest for biotechnological applications (Ferreira *et al*. 2010; Pereira *et al*. 2012). The bioactive molecules comprise high molecular weight compounds, mainly polysaccharides, and low molecular weight secondary metabolites (de Silva *et al*. 2013). Polysaccharides (especially β-glucans) are the best known and most potent mushroom-derived substances, with antitumor and immunomodulatory effects, thus acting as biological response modifiers (BRMs) by improving the host immune system (Chan *et al*. 2009; Chen & Seviour 2007; Wasser 2002; Zhang *et al*. 2007). The vast structural diversity of mycochemicals (phenolic compounds, terpenes, lactones, steroids, alkaloids, among others) provides unique opportunities for discovering new drugs that target and modulate molecular and biochemical signal transduction pathways (Chang & Wasser 2012; Patel & Goyal 2012; Zaidman *et al*. 2005). Some species possess a variety of bioactive compounds and therefore may be able to produce enhanced pharmacological effects. The best example is *Ganoderma lucidum* (Curtis) P. Karst., which contains not only more than 120 different triterpenes but also polysaccharides, proteins and other bioactive molecules (Wasser 2010b).

Owing to this plethora of useful bioactive compounds, mushrooms represent a growing segment of today's pharmaceutical industry.

Better insight into the different roles of multiple active compounds and the mechanisms underlying their biological action will accelerate commercial production of pharmaceuticals for therapeutic applications. Asian countries have a head start in the study of medicinal mushrooms compared to the rest of the world, and Western medicine still has a lot to learn from Eastern practices (Paterson & Lima 2014). As presented later in this chapter, several immunoceutical polysaccharides have been developed for clinical and commercial purposes in Japan, Korea, and China. For instance, the Chinese pharmacopeia lists more than 100 mushroom species for medicinal use, and fungal polysaccharide extracts have been used for over three decades as an adjuvant to cancer radio- and chemotherapy (El Enshasy & Hatti-Kaul 2013; Kidd 2000; Martel *et al*. 2014).

Ongoing research projects are aiming to promote mushrooms as a new generation of "biotherapeutics" (Patel & Goyal 2012). Given that only about 10% of mushroom biodiversity has been studied so far (see Chapter 2), and few of them have been characterized with regard to health benefits, it is likely that new active compounds will be discovered in the future (Hawksworth 2012). Particularly in tropical areas, 22–55% (in some cases up to 73%) of mushroom species have not yet been described (Bass & Richards 2011).

Medicinal mushroom science has been recognized as a successful multidisciplinary new branch of science which has experienced great progress in the last 30 years. As a consequence, around 400 clinical trials have been performed to evaluate the effects of medicinal mushrooms in various diseases and more than 50 000 scientific studies and 15 000 patents on medicinal mushrooms have been produced so far (Wasser 2014).

This chapter will summarize the available information and reflect the present state of mushroom use for developing functional foods, drugs, and nutraceuticals. These prospects are expected to provide new avenues for upgrading mushrooms from functional food to translational mushroom medicine.

5.2 A Window into the "Garden" of a Novel

Class of Products

The Chinese have an ancient saying which highlights the concept that medicine and food have a common origin. At the intersection between food, nutrition, and medicine and encouraged by growing concerns about the impact of diet on health and efforts to achieve "optimal nutrition," a rich "garden" of terms has emerged, for many of which there are no absolute definitions accepted by the scientific community. In this section, we will try to open a window into this puzzle in order to provide a comprehensive perspective on the contemporary uses of mushrooms in the context of this book.

Most mushroom-derived preparations find use not as pharmaceuticals ("real" medicines) but rather as a novel class of products with different names: food supplements, tonics, functional foods, nutraceuticals, phytochemicals, mycochemicals, biochemopreventives, and designer foods (Chang 2009; Wasser & Akavia 2008). Our starting point will be the functional foods and nutraceuticals, a growing field in food science seeking alternatives to improve personal health and reduce healthcare costs. According to the International Life Sciences Institute of North America (ILSI), functional foods are "foods that by virtue of physiologically active food components consumed as part of the usual diet provide health benefits and/or reduce the risk of chronic diseases beyond basic nutritional functions" (Coles 2013). Such foods range from traditional foods possessing demonstrated physiological benefits as well as processed foods, e.g. fortified with added or concentrated ingredients to functional levels (Betoret *et al.* 2011; Prakash *et al.* 2014).

The term "nutraceutical" was coined from "nutrition" and "pharmaceutical" in 1989 by Dr Stephen DeFelice and is defined as "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease." Based on this definition, a functional food would be a kind of nutraceutical (Keservani *et al.* 2010) and in some countries the two terms are used interchangeably.

In the case of mushrooms, the terms "nutraceutical" and "functional food" are synonymous (Chang & Buswell 1996, 2003). In the general context of this book, including wild edible plants and nuts, we will discuss "mushroom nutraceuticals" in

correspondence with the Health Canada definition describing them as products isolated or purified from foods generally sold in "pharmaceutical forms" of pills, capsules, and liquids, not usually associated with food. A nutraceutical is demonstrated to have a physiological benefit or provide protection against chronic disease (Mahabir & Pathak 2013). Thus, nutraceuticals could be found in many products emerging as "dietary supplements," comprising ingredients obtained from food, plants, and mushrooms (fungi) that are taken without further modification, separately from foods for their presumed health-enhancing benefits. Therefore, they may be classified as a category between foods and drugs (Wasser & Akavia 2008).

"Phytochemicals" are specific types of nutraceuticals and comprise the naturally occurring, biologically active compounds found in plants which have capabilities of inhibiting various diseases, as part of the antioxidant defense molecules among other physiological actions on the human body. Important phytochemicals are secondary metabolites such as phenolic compounds, sterols, and alkaloids. Phrases like "chemopreventive agents" are sometimes used to describe phytochemicals thought to reduce risk for certain types of cancer (Jabeen *et al*. 2014). Analogically, "mycochemicals" refers to the untapped metabolites from mushroom fungi that can be used as nutraceuticals and as new life-saving drugs (Patel *et al*. 2012). Similar to "phytopharmaceuticals," the resulting drugs should be considered as "mushroom pharmaceuticals" (Lindequist 2013).

In the mushroom science community, the term "nutriceutical" is also an accepted definition emerging from the recognition of numerous biological activities of mushroom products. A "mushroom nutriceutical" is a refined/partially refined extract or dried biomass from either the mycelium or fruiting body of a mushroom, which is consumed in the form of capsules or tablets as a dietary supplement (DS) (not a food) and has potential therapeutic applications (Chang & Buswell 1996, 2003; Chang & Miles 2004). According to Wasser and Akavia (2008), mushroom-based products can serve as a diverse and superior class of dietary supplements. Regular intake of medicinal mushroom preparations may enhance the immune response of the human body, thereby increasing resistance to disease. Acting as immunopotentiators,

these mushroom preparations modify host biological responses and therefore, they are also known as biological response modifiers (BRMs) (Chang 2009; Wasser 2014; Wasser & Weis 1999). Moreover, several classes of mushroom bioactive substances having immunotherapeutic efficacy when taken orally can be considered as immunoceuticals (Kidd 2000; Petrova *et al.* 2005).

Although our garden seems like an intricate labyrinth, the truth is that edible and medicinal mushrooms as well as mushroom products have definitively arrived (Chang & Wasser 2012). The next questions are:

- How can humans use mushrooms as innovative resources for a healthy lifestyle and in preventive and curative medicine?
- What defines a particular use?
- Are mushroom products "magic" like the foods of "Alice in Wonderland"?

5.3 Main Uses of Edible Medicinal Mushrooms in the Age of Human Health Crises

It is well known that we live in an age of human health crises. This is where the role of edible and medicinal mushrooms with their products has become important (Chang & Buswell 2003; Cheung 2008). Nowadays, interest in biotechnological cultivation of basidiomycete mushrooms is related to the increasing demand for mushroom-based biotech products in the pharmaceutical, food, and cosmetic industries (Badalyan 2014). The physiological functions of mushrooms can be described by the pyramid model suggested by Chang and Wasser (2012). In this model, human health may be divided into three states: health, subhealth, and illness. Mushrooms themselves can be used as a food to promote a healthy state; pure refined products can be used as medicine for ill health, and crude extract products can be used as dietary supplements (nutraceutical for our purpose) for a subhealthy state, as well as for both healthy and ill states.

Thus, mushrooms are not only food but are the raw material for development of functional food and dietary supplements (nutraceuticals). Mushrooms as functional food can help in the early intervention of subhealthy states and may prevent the consequences of life-threatening diseases. The ideal strategy is subhealthy intervention and prevention rather than cure of chronic nontransmissible diseases by reverting to traditional knowledge as a source of chemopreventive food and nutraceuticals. Further, the quality of life of those who are on lifelong therapeutic drugs may be enhanced by using functional molecules from mushrooms (Vikineswary & Chang 2013). When used as drugs, mushroom products can supplement other treatments and complement modern medicine (Chang & Wasser 2012; Wasser 2014).

Between 80% and 85% of mushroom products are taken from fruit bodies either collected in the wild or grown commercially, and the resulting products are considerably diverse and unpredictable. Only 15% of all products are based on extracts from mycelia and a small percentage are obtained from culture filtrates (Barros *et al.* 2007; Lindequist *et al.* 2005). One main prerequisite to using mushrooms as drugs, nutraceuticals or for other purposes is its continuous production in high amounts and at standardized quality. In addition, safety of mushrooms and their products should be verified and proven as thoroughly as possible (Chang & Wasser 2012). In the opinion of Chang (2001), mycelial products are the "wave of the future" because they ensure standardized quality and year-round production. Thus, submerged liquid fermentation can provide more uniform and reproducible biomass and may provide valuable medicinal products (Suárez & Nieto 2013). However, fruiting bodies obtained under good manufacturing practice (GMP) can also be used in the formulation of consistent and safe mushroom products such as functional foods, nutraceuticals, and biologically active compounds (Morris *et al.* 2014a).

As mentioned above, the range of human states in which mushroom-derived products can be used is broad. Therefore, in this section, an attempt will be made to dissect and distinguish the importance and uses of mushrooms as part of a modern healthy lifestyle by passing from cuisine to clinical applications.

5.3.1 Mushrooms as Functional Foods: A Paradigm of Integrating Tradition and Novelty

In agreement with the notion that prevention is better than cure, functional foods based on medicinal mushrooms have gained popularity for their high nutritive and medicinal values (Chang & Miles 2004; Mane *et al*. 2014; O'Neil *et al*. 2013). Generally, edible mushrooms possess all three desired properties of food: nutrition (see Chapter 3), taste, and physiological functions (Chang & Buswell 2003; Chang & Wasser 2012).

Over a 15-year period (1997–2012), the global per capita consumption of mushrooms increased from about 1 kg/year to over 4 kg/year, with *Agaricus*, *Pleurotus*, *Lentinula*, *Auricularia*, and *Flammulina*, the so-called "high five," accounting for 85% of the world's mushroom supply (Royse 2014). Commercial cultivated mushrooms are readily available fresh, frozen or canned and they are useful and versatile ingredients that can easily be added to many dishes such as pizzas, casseroles, and salads (Stamets 2002). For example, in Japan fresh and dried shiitake (*Lentinus edodes* (Berk) Singer) is used in medicinal mushroom dishes – "Yakuzen." These dishes can be prepared in many ways: boiled, grilled, skewered, or on aluminum foil with different types of seasoning. Concentrates, obtained from whole fruiting bodies or powdered mushrooms, are used as drinks (Wasser 2010c). Mane *et al*. (2014) reported an improvement in nutritional quality and therapeutic properties of meal items through the addition of fresh or fried oyster mushroom *Pleurotus sajor-caju* (Fr.) Singer without affecting its acceptability.

It is important to note the potential relevance of new species of culinary-medicinal mushrooms cultivated recently at commercial scale, e.g. *Flammulina velutipes* (Curt.: Fr.) P. Karst., *Tremella* spp., *Coprinus comatus* (O.F.Mull.: Fr.) Pers., *Hypsizygus* spp., *Dictyophora* spp., and *Hericium erinaceus* (Bull.: Fr.) Pers. among others (Chang & Wasser 2012). Wild mushrooms, for example, the nutritional and chemical (antioxidant) inventory of Portuguese edible mushrooms in different habitats (Pereira *et al*. 2012), also deserve interest for the development of functional

foods.

Several mushrooms are helpful in human ailments because they possess many typical pharmacological features, such as metabolic activation, bioregulation (maintenance of homeostasis and immune balance), prevention/control of intoxication, decreasing cholesterol levels, as antioxidants with rejuvenating and energy-boosting properties, and their role in the prevention and improvement of life-threatening diseases such as cancer, neurodegenerative disorders, diabetes, and metabolic syndrome (Lindequist *et al.* 2005; Patel *et al.* 2012; Roupas *et al.* 2012). In view of these properties, mushrooms have been considered as "the new superfood" or "the choicest food of nutritionists" (Mane *et al.* 2014).

Much more research is needed on the bioactive components in mushrooms to determine their biological responses in humans. Promising evidence suggests that ergothioneine, vitamin D, β-glucan, and selenium offer positive effects for immune function, intestine function, and weight management (Feeney *et al.* 2014). Information about the proximate composition and energy as well as mushroom mycochemicals is of great interest as both fruiting bodies and mycelia could be used as functional foods and/or as a source of functional ingredients. Thus, the benefits of mushrooms in human nutrition are growing as more research is undertaken to validate traditional claims.

5.3.1.1 Proven Functional Properties

Improvement of Digestive Function

Mushrooms contain dietary fibers, including β-glucans, chitin, and heteropolysaccharides (pectinous substances, hemicelluloses, polyuronides, etc.), as much as 10–50% in the dried matter (Wasser & Weis 1999). Benefits of insoluble dietary fiber include reduction of bowel transit time, prevention of constipation, and reduction in risk of colorectal cancer. Concerning soluble dietary fibers and especially β-(1,3),(1,6)-D-glucans, health benefits

include lowering of blood cholesterol, reducing hyperglycemia and hyperinsulinemia in relation to the control of diabetes mellitus, reduction of risk factors for degenerative diseases such as cardiovascular disease, cancer, hypertension, and promotion of the growth of beneficial gut microflora (as a prebiotic) (Jacobs *et al*. 2009; Laroche & Michaud 2007).

Constipation is one of the most prevalent gastrointestinal complaints and high fiber intake is recommended as an initial therapy. Ear mushrooms (*Auricularia*) are known to have higher fiber content (by 50%) than other mushroom varieties. In patients with functional constipation, fiber supplements using ear mushrooms have been shown to significantly improve constipation-related symptoms without serious side-effects (Kim *et al*. 2004).

Synytsya *et al*. (2009) reported that the fruit bodies of *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. and *P. eryngii* (DC.) Quél. contain significant amounts of β-glucans, which are components of both insoluble and soluble dietary fibers. The stems are a better source of insoluble dietary fibers and glucans than the gastronomically attractive pilei, and therefore the stems can be used for the preparation of biologically active polysaccharides utilizable as functional foods. Mushroom polysaccharides can stimulate the growth of colon microorganisms, e.g. acting as prebiotics. Potential prebiotic activity of glucan extracts L1 (water soluble) and L2 (alkali soluble) isolated from stems of *P. ostreatus* and *P. eryngii* was tested using probiotic strains of *Lactobacillus*, *Bifidobacterium*, and *Enterococcus*. These probiotics showed different growth characteristics dependent on extract used and strain specificity. This exploitation of fruit body extracts extends the use of *P. ostreatus* and *P. eryngii* for human health.

Interactions between the host and its microbiota are increasingly recognized to be critical for health. Rapid and reproducible changes in human gut microbiota were evidenced in an interventional randomized clinical trial conducted with healthy volunteers treated for 14 days with a *Trametes versicolor* (L.: Fr.) Lloyd extract at doses of 1200 mg, three times daily (Beth Israel Deaconess Medical Center, NCT 01414010, http://clinicaltrials.gov/ct2/results?term=mushroom).

Antioxidant Properties

Mushrooms packed with a wide array of bioactive components are excellent antioxidants and antiinflammatory agents which may help to prevent the occurrence and aid the treatment of chronic diseases including heart disease and various cancers (Vikineswary & Chang 2013). Primary metabolites, including enzymes such as glucose oxidase, superoxide dismutase, peroxidases, and laccases, may prevent oxidative stress (Chang & Wasser 2012; Wasser 2010a). In addition, some common widely consumed edible mushrooms have been found to possess antioxidant activity (see Chapter 4), which is well correlated with their total phenolic content. Phenolics can act as free radical inhibitors (chain breakers), peroxide decomposers, metal inactivators or oxygen scavengers and thus delay food spoilage and oxidative damage in the human body (Asatiani *et al*. 2010).

The ability of preparations from *Pleurotus ostreatus*, *Agaricus bisporus* (J. Lge) Imbach and *Ganoderma lucidum* (Curt.: Fr.) P. Karst to prevent oxidative damage of DNA has been established (Jose *et al*. 2002).

Palacios *et al*. (2011) investigated the antioxidant properties of eight types of edible mushrooms (*Agaricus bisporus*, *Boletus edulis* Bull., *Calocybe gambosa* (Fr.) Donk, *Cantharellus cibarius* Fr., *Craterellus cornucopioides* (L.) Pers., *Hygrophorus marzuolus*, *Lactarius deliciosus* (L.) Gray, and *Pleurotus ostreatus*). Homogentisic acid was the free phenolic acid significantly present in all mushrooms although the content varied considerably among the analyzed species. Flavonoids, such as myricetin and catechin, were also detected in the mushrooms studied. The antioxidant properties were evaluated by monitoring linoleic acid autoxidation, and all the species showed inhibition, with *C. cibarius* being the most effective (74% inhibition) and *A. bisporus* the species with lowest antioxidant activity (10% inhibition).

The oyster mushroom, *P. ostreatus*, has potent antioxidant activity by virtue of its scavenging hydroxyl and superoxide radicals, inhibiting lipid peroxidation, reducing power on ferric ions, and chelating ferrous ions. *P. ostreatus* also exhibits good *in vivo* antioxidant activity by reducing lipid peroxidation and enhancing the activities of enzymatic and the levels of nonenzymatic antioxidants. The antioxidant principles identified, such as ascorbic acid, α-tocopherol, β-carotene and flavonoid compounds (rutin and chrysin), possibly contributed to the observed effects (Jayakumar *et al.* 2011). Phenolic compounds were detected in five extracts obtained from fruit bodies of *Pleurotus* spp., obtained with solvents of different polarity; however, the highest levels were found in polar extracts (water and ethanol) with values of 138.4 and 86.37 mg/100 g dry base, respectively (Beltrán *et al.* 2013).

In addition to their total phenolic content, the antioxidant activity of mushrooms was also found to be due to their polysaccharide content. Khan *et al.* (2014) evaluated the antioxidant (lipid peroxidation inhibition) and functional (swelling power, fat binding, foaming, and emulsifying properties) properties of β-glucans extracted from edible mushrooms *A. bisporus*, *P. ostreatus*, and *Coprinus atramentarius* (Bull.) Fr. The glucan from *C. atramentarius* showed better antioxidant and functional properties compared to those from *A. bisporus* and *P. ostreatus*. Fungal pigment melanin also possesses antioxidant, immune-modulating, antimutagenic, and radioprotective properties (Badalyan 2014).

Selenium has also received increasing attention as a possible cancer preventive trace mineral, possibly through antioxidant protection and/or increased immune function. Mushrooms accumulate selenium based on their growing medium and provide more selenium than other foods in the fruit and vegetable group (Sadler 2003). Using the vacuum impregnation technique, Cortés *et al.* (2007) developed a product with functional characteristics by means of fortification of *P. ostreatus* mushroom with calcium, selenium, and ascorbic acid. Fortification levels for Ca and Se of 7.3% and 42.3% of the Daily Recommended Intake (DRI)/100 g of fresh mushroom, respectively, were obtained. At the beginning of

storage at 4 °C, the ascorbic acid content was 40% of the DRI/100 g of fresh mushroom. In another study, Mao *et al*. (2014) purified and evaluated the antioxidant activities of selenium-containing proteins and polysaccharides in the Royal Sun mushroom, *Agaricus brasiliensis*.

The antioxidant properties displayed by edible mushrooms as functional foods are also closely associated with their antimutagenic, antigenotoxic, radioprotective, and antiaging effects. Moreover, Naveen and Anilakumar (2014) reported that the antifatigue property of *A. bisporus* was supported through decreased levels of lipid peroxidation in tissue and also proposed the development of a fermented yogurt product using an *A. bisporus* extract.

It is important to highlight that mushrooms are generally cooked or processed into various culinary dishes industrially or at home. Cooking processes bring about a number of changes in their physical characteristics and chemical composition, including an effect on antioxidant activity. Arora (2014) stated that, in general, frying does not affect antioxidant activity but boiling and microwave cooking deplete the radical scavenging ability of *A. bisporus*, *Calocybe indica*, *Volvariella volvacea* (Bull.: Fr.) Sing, *Lentinula edodes*, and *P. ostreatus*.

Improvement of Blood Lipid Profile and Lower Risk of Cardiovascular Disorders

Mushrooms may be able to improve cardiovascular disease risk through their ability to reduce blood cholesterol levels. The results of numerous studies indicate that mushrooms are a valuable source of statins (Endo 2004), which inhibit the activity of the key enzyme in cholesterol synthesis, hydroxyl-methyl-glutaryl-CoA reductase (HMG-CoA reductase). The best known edible higher basidiomycetes for potential production of lovastatin are species of the genus *Pleurotus* and the highest content was found in the fruiting bodies of *P. ostreatus* (Gunde-Cimerman & Plemenitas 2001).

It is known that shiitake mushroom (*L. edodes*) is able to lower blood cholesterol and lipids in animals and humans via a factor known as eritadenine (also called "lentinacin" or "lentysine"). Apparently, eritadenine reduces serum cholesterol in mice, not by inhibition of cholesterol biosynthesis but by acceleration of the excretion of ingested cholesterol and its metabolic decomposition. For many patients (60 years of age or older) with hyperlipidemia, consuming fresh shiitake mushroom (90 g/day for seven days) led to a decrease in total cholesterol blood level by 9–12% and triglyceride level by 6–7% (Hobbs 2000). Although feeding studies with humans have indicated positive effects, further research is needed.

In addition to the improvement in blood lipid profile, the cardioprotective role of mushrooms is also related to their antithrombotic activity (antiaggregatory action on blood platelets), including nucleic acid components of *L. edodes* (Kabir & Kimura 1989) and a blood pressure-lowering effect (*e.g.* cardioactive proteins of *V. volvaceae* (Yao *et al.* 1998) and antihypertensive angiotensin I-converting enzyme inhibitory peptides from *Pleurotus cystidiosus* O.K. Mill. and *Pleurotus cornucopiae* (Paulet) Rolland (Ching *et al.* 2011). A new glycoprotein (Fraction SX) obtained from *Grifola frondosa* (Dicks.) Gray (Maitake) helps to maintain healthy cardiovascular function (Zhuang & Wasser 2004).

In China, more than 40 patents use *Tremella* as the base for food products. It can be made into a mushroom tea with the health-promoting functions of nourishing the kidneys, preventing coagulation, lowering blood pressure and prolonging life, and is a multifunctional nutrient liquid that lowers fat and cholesterol levels in blood, prevents cancer, and increases the number of leukocytes. A unique feature of *Tremella* mushrooms is that its most often mentioned medicinal properties depend on glucuronoxylomannans contained in fruiting bodies, or those produced in pure culture conditions. In particular, the hypocholesterolemic actions may be attributable to the high molecular weight anionic charged polysaccharides, involving the suppression of cholesterol absorption from the digestive tract (Reshetnikov *et al.* 2000). These bioactive materials may be

beneficial for applications in the medicinal food industry.

Improvement of Glucose Homeostasis and Antidiabetic Effect

Some protective effects of mushrooms as functional foods have been investigated, *in vitro* and *in vivo*, while some clinical trials have confirmed their therapeutic implications as an effective alternative treatment for type 2 diabetes mellitus (Deepalakshmi & Mirunalini 2014). This effect appears to be mediated via mushroom polysaccharides (possibly both α- and β-glucans) via a direct interaction with insulin receptors on target tissues, although this mechanism remains to be confirmed (Roupas *et al*. 2012).

A randomized, double-blinded, and placebo-controlled clinical trial ($n = 72$) showed that *A. blazei* Murill supplementation in combination with metformin and gliclazide improved insulin resistance in these subjects. An increase in adiponectin concentration after *A. blazei* extract consumption for 12 weeks may be the relevant mechanism (Hsu *et al*. 2007).

Jayasuriya *et al*. (2012) reported that long-term consumption of *P. ostreatus* and *P. cystidiosus* as a functional food appears to be effective for glycemic control. The study evaluated the effect of a suspension, made with powdered mushrooms, on the fasting and postprandial serum glucose levels in healthy volunteers at a dose of 50 mg/kg body weight, followed by a glucose load. Reductions in the fasting serum glucose levels for *P. ostreatus* and *P. cystidiosus* groups were 6.1% and 6.4%, respectively and the postprandial glucose reductions were 16.4% and 12.1%. Antihyperglycemic activity was demonstrated with a water-soluble polysaccharide from *P. citrinopileatus* fermentation broth. The polysaccharide was effective in lowering blood glucose levels in diabetic rats (Hu *et al*. 2006). Additionally, the *in vitro* and *in vivo* antidiabetic activity of *Calocybe indica* suggests its therapeutic potential for the prevention and control of diabetes as an easily accessible source of a natural antidiabetic functional food (Rajeswari & Krishnakumari 2013).

Other results indicated that *Tremella mesenterica* Schaeff. (fruiting bodies, submerged culture biomass and tremellastin, an acidic glucuronoxylomannan polysaccharide) might be developed as a potential oral hypoglycemic agent or functional food for diabetic patients and those with high risk for diabetes mellitus (Lo *et al*. 2006). *Tremella* constitutes the major part of functional foods, having pronounced medicinal properties, with existing patents for hyperglycemia suppressants in the form of food or drink (Reshetnikov *et al*. 2000).

Mushroom β-glucans, as soluble dietary fiber, have been gaining interest as a food ingredient due to their beneficial role in maintaining blood sugar balance via blood sugar lowering effects, elevation of plasma insulin levels, and the enhancement of cellular insulin sensitivity; they also have been shown to help in dyslipidemia, obesity, and metabolic syndrome (El Khoury *et al*. 2012). Research into mushroom antiobesity potential conducted in men and women who were overweight or obese (n = 73) revealed a significant loss in body weight, body mass index (BMI), and waist circumference during the six months of the trial in those consuming the mushroom diet (substitution of 8 oz (227 g) of fresh mushrooms for 8 oz of meat three times/week) compared with baseline (Poddar *et al*. 2013).

Enhancement of Immune Function and Lower Risk of Certain Tumors

Edible mushrooms with functional properties have long been suggested to possess immunomodulatory effects (Lindequist 2013; Wasser & Weis 1999). It was stated in the *Ri Yong Ben Cao* (1620), written by Wu-Rui of the Ming dynasty, that “shiitake accelerates vital energy, wards off hunger, cures colds, and defeats body fluid energy” (Wasser 2010c). Many of these effects are related to the immune system and recent investigations have found evidence of the health promotion abilities associated with mushroom consumption, including antiviral, antibacterial, antifungal, and antiparasitic effects (Tejera *et al*. 2013).

Many, if not all, basidiomycete mushrooms contain biologically

active polysaccharides in fruit bodies, cultured mycelium, and culture broth. Polysaccharides are the most potent mushroom-derived substances with antitumor/immunomodulating properties. These polysaccharides are of different chemical composition, with most belonging to the group of β-glucans having β-(1,3) linkages in the main chain and additional β-(1,6) branches needed for their antitumor action. Most of the clinical evidence for immunomodulating and antitumor activities comes from the commercial polysaccharides, such as lentinan (from *L. edodes*), PSK (krestin) (from *Trametes versicolor*), and schizophyllan (from *Schyzophyllum commune* Fr.: Fr.) (Chang & Wasser 2012; El Enshasy & Hatti-Kaul 2013; Wasser 2002). The use of these mushroom polysaccharides as drugs will be discussed in section 5.3.3, and in this section the benefits of food products based on whole mushrooms or foods supplemented with β-glucans to support our immune system will be the focus of attention.

Fungi β-(1,3)-glucans are traditionally part of the Japanese diet, in which whole mushrooms are eaten. The consumption of fresh mushrooms was found to increase anti-β-glucan antibodies in the serum of humans; it was also suggested to provide better defense against pathogenic fungi (Ishibashi *et al*. 2005). In addition, dietary intakes of *A. bisporus* (fresh) and *L. edodes* (dried) mushrooms and green tea combine to reduce the risk of breast cancer in Chinese women (Zhang *et al*. 2009). Although many patents have been published claiming immunopotentiator effects of β-glucans in functional foods, in some cases β-glucan is incorporated in such a low quantity that the real health benefit is difficult to determine (Laroche & Michaud 2007).

Two types of hydrogels of β-D-glucan, pleuran (from *P. ostreatus*) and lentinan, have been added to yogurts, natural, sweetened, flavored or with fruit, to increase their bioactivity. The application of both hydrogels to yogurts had no negative influence on the sensory acceptability of the products and all samples maintained very good quality during the whole storage period. The regular daily consumption of such dairy products could contribute to the reduction of relapsing or chronic infectious as well as autoimmune and oncological diseases, especially in more risky age groups (children and older people) (Hozová *et al*. 2004).

Wild edible BaChu mushroom (*Helvella leucopus* Pers.), grown in Xinjiang Province, China, can be used in the treatment of leukocytopenia, and reduced immunity due to chronic hepatitis and radiochemotherapy. It also has a preventive role for AIDS. BaChu mushrooms are reported to enhance the phagocytosis ability of leukocytes, lymphocyte conversion ratio, and antibody titer (Meng *et al*. 2005). BaChu mushroom crude polysaccharides have been used in a processing technology for obtaining a beverage mixed with water and fresh juice. This juice recipe has more than 14 000 IU of vitamin A and over three times the vitamin C content of an apple (Hou *et al*. 2008).

Bioactivity analyses present a possible direction for developing reliable functional foods based on whole shiitake or food supplemented with isolated lentinan. The consumption of *L. edodes* has been associated with the proliferation, activation, and modification of memory and naive innate immune cell populations (Stanilka *et al*. 2013) and it modulates human immune function by altering cytokine secretion (Dai *et al*. 2013).

Nanotechnology has shown great potential for improving the extraction effectiveness of bioactive compounds in functional foods. For example, a new method was developed for nanoparticle extraction of water-soluble β-glucans from mushrooms (sparan, the β-D-glucan from *Sparassis crispa* (Wulfen) Fr., and phellian from *Phellinus linteus* (Berk. & M.A. Curtis) Teng). This "nanoknife" method could be used in producing β-glucans for the food, cosmetics, and pharmaceutical industries (Park *et al*. 2009). Nanotechnology applied to mushrooms also aims to enhance solubility, facilitate controlled release, improve bioavailability, and protect bioactive compounds during processing, storage, and distribution.

Neurogenerative Potential and Improvement of Neurodegenerative Diseases

Studies have shown that consumption of *Hericium erinaceus* (lion's mane mushroom) is associated with neurite-stimulating activity through the induction of nerve growth factor (NGF) (*in*

vitro and *in vivo*) by dilinoleoyl phosphatidylethanolamine (DLPE), hericenones C–H, and erinacines A–I. Preliminary human trials with *H. erinaceus* derivatives showed efficacy in patients with dementia in improving the Functional Independence Measure (FIM) score or retarding disease progression (Kawagishi & Zhuang 2008), while a double-blind, parallel-group, placebo-controlled trial with oral administration of *H. erinaceus* to 50–80-year-old Japanese men and women diagnosed with mild cognitive impairment reported significantly increased cognitive function scores compared to placebo during intake (Mori *et al*. 2009). Therefore, this mushroom has great potential to be developed as a functional food or nutraceutical for boosting brain and nerve health and for improvement of subhealth states related to aging and delaying neurodegeneration.

In sum, the consumption of whole edible-medicinal mushrooms or their bioactive ingredients as functional foods is a beneficial practice for preserving health. However, postlaunch monitoring is needed to establish whether functional foods are safe and effective under customary conditions of use and to assess their influence on the effectiveness of drugs and patient compliance (Coles 2013). The development of new functional foods from mushrooms is increasingly challenging. It remains to be determined how often, how much and what species or mixture of species should be consumed to bring about a desired biological response (Vikineswary & Chang 2013).

5.3.2 Mushroom Nutraceuticals

The nutraceutical revolution leads into a new era of medicine and health, in which the food industry is expected to become a research-oriented sector similar to the pharmaceutical industry (DeFelice 1995). Nowadays, different mushroom-based healthcare commercial biotech products with preventive and curative effects are available and largely consumable in the world market as nutraceuticals (dietary supplements, DS). The market for DS from mushrooms is growing and is currently valued at more than US$18 billion (representing 10% of the general market for DS) and the demand for such products is expected to increase (Wasser 2014).

For example, Aloha Medicinals Inc. (Carson City, NV), with a monthly production of 400 000 kg of finished product (equivalent to 16 million bottles of dietary supplement) is considered the largest in the world (www.alohamedicinals.com).

Numerous studies have shown that certain mushroom DSs are effective in both preventing and treating subhealth status and specific life-threatening diseases owing to the synergistic action of bioactive molecules, when regularly consumed; even in high dosages (over 150 g of fresh mushroom), they demonstrate very low toxicity. Many mushrooms or mushroom preparations traditionally taken as treatments for specific conditions are now often marketed for use as prophylactic agents (Badalyan 2014; Chang & Wasser 2012).

Mushroom-derived products are neither food (functional food) nor pharmaceuticals (drugs), because the active ingredient of most products is not a single, chemically defined compound as used in conventional drug treatments. Therefore, they may be classified as a type of DS or traditional medicine, which is a category between food and drugs (Chang & Wasser 2012). Each one is commercialized as a DS, specifying that the purpose is not to treat, diagnose, cure or prevent any disease, and they have not been evaluated by the FDA. The main types of mushroom DS products available on the market today are:

- artificial cultivated fruit body powders, hot water or alcohol extracts of these, or the same extract concentrates and their mixtures
- dried and pulverized preparations or the combined substrate, mycelium, and mushroom primordial after inoculation of edible semisolid medium (usually grains)
- biomass or extracts from mycelium harvested from submerged liquid culture grown in a bioreactor
- naturally grown, dried mushroom fruit bodies in the form of galenic formulations like capsules or tablets
- spores and their extracts (Chang & Wasser 2012; Lindequist 2013; Llauradó *et al*. 2013; Morris *et al*. 2011; Wasser & Akavia 2008).

Data regarding the dosage to be used are controversial; the

suggested dosages vary widely due to various forms and formulations. Although the fresh form can be a valuable dietary supplement, the quantities one would require for therapeutic doses are so great that its consumption could cause digestive upset. According to traditional Chinese medicine, the standard dose of the mushroom dried fruiting bodies per day in different forms (tablets, capsules, liquid extracts, etc.) must be equivalent to about 100–150 g of fresh mushroom material. Numerous clinical trials have established that six capsules (three capsules two times per day or two capsules three times per day), of 500–1000 mg each (biomass or extracts), is the accepted dosage of mushroom preparations (Wasser 2014).

We illustrate this with shiitake mushroom, which is prescribed in various forms. It may be ingested as a sugar-coated tablet, capsule, concentrate, powdered extract, syrup, tea, or wine. Tablets are usually made from a dried water extract of the mycelia or fruiting bodies because drying concentrates the lentinan and other active principles. Standardized extracts are also available, and they are preferred because the amount of lentinan present is certified and clearly stated on the bottle. The standard dose of the dried fruiting body in tea or in mushroom dishes is given as 6–16 g, equivalent to approximately 60–160 g of fresh fruiting bodies. The dosage, usually in the form of a 2 g tablet, is 2–4 tablets/day (Stamets 2002; Wasser 2010c).

A brief overview of mushroom nutraceutical products is provided in Table 5.1.

Table 5.1 Overview of some mushroom nutraceutical products and their health effects.

Product	**Content**	**Observations**
Aloha Medicinals Inc. (www.alohamedicinals.com)		
Organic *Cordyceps sinensis*™ (525 mg)	*C. sinensis alohaensis* hybrid strain (US and international patents)	50/50 mixture of hybrid *Cordyceps* and CS-4 *Cordyceps*. This product is often

		combined with *Agaricus blazei*. *Tru-Cordyceps*™
Immune-Assist™ Critical Care Formula (500–mg)	*A. blazei:* 58.5% β-(1,3)-(1,6)-D-glucan; *C. sinensis:* 30% β-glucan and deoxiadenosine and other nucleosides; *G. frondosa:* 28% β-glucan (fraction D); *L. edodes:* 40% β-glucan (lentinan) and α-glucan (KS-2); *C. versicolor:* 40% β-glucan (including polysaccharides P and K); *G. lucidum:* 40% β-(1-3)-(1-6)-D-glucan and triterpenoids	This product has proven a significant reduction of the adverse effects induced by radio- and chemotherapy in clinical trials, including appetite loss, nausea, low energy status, among others.
Immune-Assist 24/7 (500 mg)	*A. blazei, C. sinensis, G. frondosa, L. edodes, C. versicolor, G. lucidum* (similar to the former formulation) plus hybrid *Cordyceps* and a green tea-derived substance	This formula has proven to be useful in HIV/AIDS patients after clinical trials Dosage: 3 tabs/day with meals
GanoSuper™	Concentrated Reishi extracts. Made from four different strains of Reishi – Black, White, Red and	A concentrated extract for people who want a fully water-soluble form of Reishi for use in

	Purple	their coffee or tea. It is manufactured so as to make it fully water soluble so opened capsules can be dissolved directly into the coffee or other hot drink
Levolar Forte™ (750 mg)	Extract of *C. sinensis*, CS4 (from *C. sinensis*), fraction D of *G. frondosa*, extract of *Coprinus comatus*, full-spectrum *Cordyceps sinensis*, cinnamon extracts, and biotin	Specifically designed for compensating the symptoms of diabetes mellitus and fragile X syndrome Dosage: 4 tablets/ day for 2 weeks
Pharmaceutical Mushrooms (www.nwbotanicals.org)		
Eighth Element™ (500 or 600 mg)	*Cordyceps sinensis*	Increase in cellular energy in about 28.8% Dosage: 2 capsules/ day
Maitake (500 mg)	*Grifola frondosa* (contains a diversity of β-glucans)	Potent immunomodulating effect. It stimulates T cell production and is recommended for immunodeficiencies
Purica-Immune FX (250 mg)	*A. blazei, C. sinensis, G. frondosa*,	Rich in β-glucans, potent immunopotentiators,

	L. edodes, *C. versicolor*, *G. lucidum*, Nutricol™ (bioflavonoid concentrate)	and antioxidant bioflavonoids
Hep-Assist (500 mg)	Hot water extracts and ethanol precipitates of *L. edodes, A. blazei, G. frondosa, C. versicolor, G. lucidum*, and two *C. sinensis* extracts (one from mycelium and other from the culture broth)	The concentrated mixture of 200 β-glucans and nucleosides from 6 different species of mushrooms turns this formula into a valuable adjuvant product in the treatment of hepatitis B and C
Zhejiang Fangge Pharmaceutical and Healthcare Products Co. Ltd. (http://mushroom.en.alibaba.com)		
China's largest edible and medicinal mushroom processing enterprise. The company supplies mushroom powders, extracts (polysaccharides), supplements, and finished products (capsules and tea bags) from: *Grifola*		

frondosa; Lentinus edodes, Ganoderma lucidum; Agaricus blazei; Cordyceps sinensis; Hericium erinaceus; Coriolus versicolor; Poria cocos; Polyporus umbellatus; Pleurotus ostreatus; Flammulina velutipes; Coprinus comatus, Pleurotus citrinopileatus; Agrocybe aegerita; Agaricus bisporus; Tremella fuciformis; Auricularia auricula; Marasmius androsaceus; Phellinus igniarius; Phaeoporus obliquus;Antrodia cinamomea; Auricularia polytricha		
FineCo. Ltd. (www.fineco.net)		
Fine-Agaricus® Gold	Highly concentrated micropowder; active ingredients, protein-bound polysaccharides (100% *Agaricus* mushroom	Effective against several cancers by enhancing the immune system. It has a powerful balancing effect on many physiological

	polysaccharides)	functions and has been effective for treating chronic diseases
Fine-Mesima P®	Micropulverized powder of dried *Phellinus linteus* mushroom. Contains *P. linteus* polysaccharide 50%, dextrin 50%	Information not available
Mushroom Wisdom (**www.mushroomwisdom.com/products.php**)		
Super Reishi	Contains both hot water and alcohol concentrated extracts to achieve the maximum range of beneficial constituents (β-glucans and terpenes); also enhanced with immune-boosting Maitake D-Fraction®	Believed to balance and support the body systems, including heart, lung, liver, nerve, and brain function Dosage: 4 tablets daily or 2 tablets twice a day
Breast-Mate®	*Phellinus linteus* PL-Fraction™ 1000 mg; Maitake PSX-Fraction® containing 18% glycoprotein SX-fraction 160 mg; broccoli sprout extract (4:1) 100	PL-Fraction™ possesses potent activity in maintaining healthy breast cells. Breast-Mate® also contains synergistic ingredients (SX-Fraction®, green

	mg; green tea extract (50% polyphenols) 100 mg; vitamin D_3 800 IU	tea extract, broccoli extract) Dosage: 4 tablets daily or 2 tablets twice a day
Mushroom Emperors™	*A. blazei* Murill fruiting body 120 mg; *C. sinensis* mycelium powder 120 mg; *Hericium erinaceus* fruiting body 120 mg; *G. frondosa* fruiting body 120 mg; *L. edodes* fruiting body 120 mg; *Tremella fuciformis* fruiting body 120 mg; Maitake TD-Fraction® (10% D-fraction 40 mg); Maitake PSX-Fraction® (18% glycoprotein SX-fraction 40 mg; Lion's Mane Amycenone® (hericenones 0.5%, amyloban 6%, 40 mg); *P. linteus* extract PL-Fraction™ 40 mg; *Inonotus obliquus* extract 40 mg; *C. versicolor* extract 40 mg; *Poria cocos* extract 40 mg; *G.*	Mushroom Emperors™ brings together 6 holistic mushroom powders with 8 concentrated extracts, including proprietary extracts (D-fraction, SX-fraction, and amycenone) to create a synergistic blend to help promote overall health and vitality Direction for use: 4 tablets daily or 2 tablets twice a day

	lucidum double extract 40 mg; vitamin C 80 mg	
Product 4life (www.tienda4life.mx/web/Productos.aspx)		
Transfer Factor Plus® Tri-Factor® Formula	*L. edodes, G. frondosa, Cordyceps*, β-glucans, hexaphosphate inositol, β-sitosterol, and an extract of olive leaves	Provides an optimal level of immune support, i.e. the activity of NK cells can be increased to 437%. Also benefits the cardiovascular system

We can conclude that the diversity of mushroom DSs with respect to composition/formulation items (combination of components containing in biomass, extracts or isolated fractions of different mushroom species in one preparation or only one species, combination of mushroom substances with other herbal products or pure nutraceuticals such as vitamins and minerals, etc.) is enormous. Most of these mushroom DSs containing polysaccharides function as immunomodulators. The physiological constitution of host defense mechanisms is improved, which restores homeostasis, thereby enhancing resistance to disease and in some cases causing regression. For example, products developed from biotechnologically cultivated mycelia of edible mushrooms *Hericium erinaceus* and *Tremella* spp. in combination with other natural substances possess antioxidant and immune-stimulating activity, and regulate the level of blood lipids and sugar (Khan *et al*. 2013; Standish *et al*. 2008).

In developing productive research programs for nutraceuticals, it is important to build a hierarchy of evidence for individual supplements, including understanding the essentials of product characterization (purity, active ingredients, and potential mechanisms of action), basic clinical chemistry, and subsequent

rigorous testing in the setting of clinical studies. Multiple lines of investigation can then be coordinated for enhancing the knowledge base on a product, with the goal of informing practitioners and the public on safety and efficacy of DS use (Hopp & Meyers 2010). The growing DS industry has prompted the need for international governance in establishing regulatory and standard benchmarks for the expanding world market. The scientific validation of mushroom products can help boost their credibility (Wasser & Akavia 2008).

Where should functional foods and nutraceuticals (FFN) be positioned in current guidelines as treatments for lifestyle-related diseases? FFN, similar to pharmaceutical agents, contain bioactive substances that target and modulate biological processes that foster the development of disease. FFN are likely to prove useful in both alleviating and preventing human diseases. Thus, the gap that currently exists between FFN research and the medical community needs to be closed such that FFN can be implemented into clinical guidelines for chronic nontransmissible diseases throughout all stages of therapy.

By synthesizing the benefits of both food and medicine, nutraceuticals are expanding into a wide range of areas, competing against such basic items as raw fruit and vegetables and, in some cases, cutting-edge pharmaceuticals (DeFelice 1995).

5.3.3 Mushrooms as a Significant Source of Drugs: Lessons from Wasser's Discovery Pathway

According to current categories of botanical products, medicinal mushrooms can serve as "botanical drugs" or "real drugs." Botanical drugs are complex extracts to be used for treatment of disease and they are clinically evaluated for safety and efficacy just like conventional drugs, but this process can be expedited because of the history of safe human use. Botanical drugs are highly but not completely characterized and are produced under the same strictly regulated conditions as conventional pharmaceuticals. Drugs (prescription drugs or over-the-counter drugs) require the most rigorous testing, including three phases of clinical testing, to

ensure safety and efficacy, and close scrutiny by the FDA and/or EFSA (Chang & Wasser 2012).

Öztürk *et al*. (2014) reported on mushroom species which were studied for their chemistry and biological activities in the last two decades. In general, the authors covered 24 types of polysaccharides including β-glucans and other complexes from 13 mushroom species; 259 terpenoid compounds including seven monoterpenes, 19 sesquiterpenes, 54 diterpenes, and 179 triterpenes from 29 mushroom species; 59 steroid compounds from 10 mushroom species; 41 phenolic compounds from 13 mushroom species; and 42 alkaloid compounds from 13 species. Therefore, it is important to develop a knowledge base for individual products, which will provide direction for further clinical investigations.

What steps should we follow to discover a myco-compound with potential as a drug? Wasser (2010a) proposed the Drug Discovery Pathway, which was specially prepared for the development of mushroom pharmaceuticals. This pathway includes nine steps:

- mushroom cultivation and biomass production
- biomass extraction
- screening of mushroom extracts
- effect of selected extracts on a target of interest
- chemical fractionation of selected extracts
- elucidation of active fractions (compounds), mechanism of action, and potency
- effect on animal models
- preclinical drug development
- clinical drug development.

Wasser's Drug Discovery Pathway gives a step-by-step guide and each phase provides recommendations for successful development of mushroom drugs, from the test tube of a mushroom collection to final clinical applications. The pathway will also open new avenues in this "central highway" because there are concerns to solve and questions to answer. Future biotechnological development, the application of modern high-tech screening, the OMICs sciences such as genomics and proteomics, research on

validated animal models, and the accurate assessment of clinical values of the candidate drug are directions for approval of mushroom products as drugs. Although Wasser's Pathway is valid for any mushroom drug candidate, in particular, it is intended to play a pivotal role in discovering the potential of low molecular weight metabolites for their use as drugs, i.e. targeting cancer.

Out of the huge diversity of activities, the most frequently sought for the majority of mushrooms is antitumor/immunomodulating activity. Those compounds able to stimulate the biological response of immune cells are being pursued for the treatment of cancer, immunodeficiencies (i.e. to protect AIDS patients against opportunistic infections) or for immunosuppression following drug treatment or surgical procedures. They are also sought for combined therapies with antibiotics and as adjuvants for vaccines (Lull *et al*. 2005; Wasser 2014). Polysaccharides are the most potent mushroom-derived substances with antitumor/immunomodulating properties (El Enshasy & Hatti-Kaul 2013; Mizuno 1999; Wasser 2002). Mushroom polysaccharides occur mostly as glucans, some of which are linked by β-(1-3),(1-6) glycosidic bonds and α-(1-3) glycosidic bonds, but many are true heteroglycans. Historically, hot water-soluble fractions (decoctions and essences) from medicinal mushrooms, i.e. mostly polysaccharides, were used as medicine in the Far East (Hobbs 2000).

Polysaccharides demonstrating remarkable antitumor and immunomodulating activity *in vivo* have been isolated from various species of mushrooms belonging to the Auriculariales, Tremellales, Polyporales, Gasteromycetideae, and Agaricomycetideae. The number of polysaccharides extracted from the fruiting body or cultured mycelium of each species is strongly dependent on the method of fractionation used, but in general, the total amount of polysaccharides is higher in fruiting bodies (Wasser 2002). In addition to their immune regulation potential, polysaccharides are useful biologically active ingredients for pharmaceutical use, such as for antiradiation, anti-blood coagulation, anti-HIV, and hypoglycemic activities (Shenbhagaraman *et al*. 2012).

One of the first reports on antitumor activities of hot water extracts

from fruiting bodies of mushrooms belonging to the family Polyporaceae (Aphyllophoromycetideae) and a few other families was published by Ikekawa *et al*. (1969), demonstrating a host-mediated effect against grafted cancer, such as sarcoma 180 in mice. After this, the first three major drugs were developed and commercialized from medicinal mushrooms; the three were polysaccharides, specifically β-glucans (krestin (PSK) and polysaccharide-peptide (PSP)) from cultured mycelia of *Trametes versicolor*, lentinan from fruiting bodies of *Lentinus edodes*, and schizophyllan (SPG, sonifilan, sizofiran) from liquid cultured broth of *Schizophyllum commune*. In addition, more than 100 types of polysaccharides with biological activities have been isolated from the fruiting body and mycelia of *Ganoderma lucidum* (e.g. ganoderan, GLPS) (Wasser 2010b). Among the most studied mushroom polysaccharides in Japan, China, Korea, Russia, and the US for immunomodulating/antitumor activities, we can mention grifolan or GRN, D- and MD-fractions (from *Grifola frondosa*), PL (from *Phellinus linteus*), PG101 (from *Lentinus lepideus* (Fr.) Fr.), CA1 β-glucan fraction and SCG (from *Sparassis crispa*), and befungin (from *Inonotus obliquus* Pers. (Fr.) Boud. et Sing.) (Chen & Seviour 2007; Kidd 2000; Lull *et al*. 2005; Zhang *et al*. 2011).

Mushroom polysaccharides are among the emerging new agents that could directly support or enhance immunotherapy, and their safety in use is important in biomedical science. More than 50 mushroom species have yielded potential immunoceuticals that exhibit anticancer activity *in vitro* or in animal models and of these, only a few have been investigated in human cancers. The β-D-glucans or β-D-glucans linked to proteins are currently the most promising class of immunoceuticals, displaying stronger immunoenhancing activity than the corresponding free glucans (Kidd 2000; Petrova *et al*. 2005; Vannucci *et al*. 2013; Wasser 2014).

A number of mushroom immunoceuticals polysaccharides have proceeded through phase I, II, and III clinical trials. Lentinan (*L. edodes*), PSK and PSP (*T. versicolor*) have been used in clinical trials with hundreds of cancer patients (stomach, colorectal, esophageal, lung, breast, nasopharyngeal, and leukemia). Other

compounds have only been assessed with a relatively small number of patients and in many cases, the standards of these trials may not meet the current Western regulatory requirements, although significant improvements in quality of life and survival of patients are reported (Paterson & Lima 2014). A number of Chinese patents on the medicinal application of lentinan administered orally (Sun & Wei 2007) or intravenously (Ma & Wang 2007) have been published. The effect of lentinan in prolonging life has been observed, especially in those with gastric and colorectal carcinoma, and this polysaccharide has been approved for clinical use in Japan for many years and is manufactured by several pharmaceutical companies (Zhang *et al.* 2011). Schizophyllan has also exerted beneficial activity for patients with head and neck cancers, recurrent gastric cancer, stage 2 cervical cancer, and advanced cervical carcinoma (Hobbs 2000).

PSK and PSP from *T. versicolor* have controlled various carcinomas in human clinical trials. In Japanese trials undertaken since 1970, PSK significantly extended survival at five years or beyond in stomach, colorectal, esophagus, nasopharyngeal, and lung (nonsmall cell types) cancers, and in a HLA B40-positive breast cancer subset. PSP was subjected to phase II and III trials in China. It significantly improved quality of life and enhanced immune status in 70–97% of patients with stomach, esophagus, lung, ovary, and cervix cancers. PSK and PSP boosted immune cell production, ameliorated chemotherapy symptoms, and enhanced tumor infiltration by dendritic and cytotoxic T cells. Their high tolerability, proven benefits to survival and quality of life, and compatibility with chemotherapy and radiation therapy make them well suited for cancer management regimens (Kidd 2000).

In clinical studies, *G. lucidum* products have been widely used as a single agent or in combination with other herbal medicines or chemotherapeutic drugs, mainly in Asian countries. However, randomized, placebo-controlled and multicenter clinical studies using *G. lucidum* alone have rarely been reported. In one randomized, placebo-controlled clinical study, 143 patients with advanced previously treated cancer were given an oral *G. lucidum* polysaccharide extract (Ganopoly) of 1800 mg three times daily for 12 weeks. The prostate-specific antigen (PSA) levels in the five

prostate cancer patients were reduced significantly, indicating that Ganopoly may have an adjunct role in the treatment of patients with advanced cancer although objective responses were not observed (Gao *et al*. 2002). A polysaccharide injection formulated from *G. lucidum* has been also developed (Jiang *et al*. 2014).

Although the maitake D-fraction is a relatively new compound, the claims of benefit are encouraging. There are a number of clinical trials in breast, prostate, lung, liver, and gastric cancers under way in the US and Japan, and several US physicians have reported good results with maitake D-fraction. Grifolan-D accomplished (>95%) cell death of prostate cancer cells *in vivo* and hindered metastatic progress, increased NK cell activity, and maintained the elevated levels of cytotoxicity for more than one year (Kodama *et al*. 2003).

Much recent research has been carried out on *Pleurotus* spp. crude extracts and isolated compounds such as polysaccharides, proteins, and other substances that possess antitumor and immunostimulatory activities (Gregori *et al*. 2007). Antitumor effects have been shown on different human tumor cell lines. From these results, POPS-1, a water-soluble polysaccharide from the fruiting bodies of *P. ostreatus*, has been considered as a potential candidate for developing a novel low-toxicity antitumor agent (Tong *et al*. 2009). A hot water mycelial extract from *Pleurotus* spp. (76.8% polysaccharides) exerted *in vitro* antiproliferative activity against human NB4 leukemia cells through apoptosis induction and cell cycle arrest in the G_2/M phase (Morris *et al*. 2014b). In light of its effects on macrophage phagocytosis and the hematopoiesis response of mice that would otherwise remain damaged by radiation and chemotherapy substances, this extract could be considered as a candidate for radio- and chemoprotective therapy (Llauradó *et al*. 2015; Morris *et al*. 2003). Used as an immunoceutical, *Pleurotus* fruiting body powder (55% polysaccharides) given orally for seven days (1000 mg/kg) to cyclophosphamide-treated mice potentiated the cellular immune response and the lymphoproliferative-stimulating index (Llauradó *et al*. 2013). Thus, *Pleurotus*-based products could be promising for clinical immunotherapy applications.

There are plenty of clinical studies proving the cancer inhibitory effects of other mushrooms such as *Inonotus obliquus*, *Phellinus linteus*, *Flammulina velutipes*, *Cordyceps sinensis* (Berk.) Sacc., etc (Wasser 2014). For example, studies conducted for antitumor activities at the National Cancer Center (Japan) demonstrated that extracts containing polysaccharides and glycoproteins prepared from *Hypsizygus marmoreus* and *F. velutipes* showed positive effects on the cachexia of advanced cancer patients. These extracts had better effects than methyl-acetoxy-progesterone in clinical response, performance status, and quality of life (Ikekawa 2005). Befungin (a multi-compound preparation containing 50% of β-(1-3),(1-6) glucans, terpenes, phenols, steroids, organic acids, and microelements) obtained from *Inonotus obliquus* was approved as an antitumor drug in Russia and reportedly successful in treating breast, lung, cervical, and stomach cancers (Badalyan 2014).

Mushroom immunoceuticals act primarily by augmenting all the key pathways of host immunity, both innate and adaptive, and signaling cascades. Due to a high potential for structural variability, polysaccharides have the necessary flexibility to affect the precise regulatory mechanisms of various cell–cell interactions (Wasser & Weis 1999). The antitumor action of polysaccharides requires an intact T cell component; their activity is mediated through a thymus-dependent immune mechanism. They activate cytotoxic macrophages, monocytes, neutrophils, natural killer (NK) cells, dendritic cells (DCs), B cells, and chemical messengers (cytokines, such as interleukins, interferons, and colony-stimulating factors) that trigger complement and acute-phase responses. Also, mushroom polysaccharides induce gene expression of various immunomodulatory cytokines and cytokine receptors (Lull *et al.* 2005; El Enshasy & Hatti-Kaul 2013; Zhang *et al.* 2007). The first step of action of these metabolites is their recognition by certain receptors located on different immune cells and activation of signal transduction pathways. It has been clarified that several β-glucan receptors mediate these activities, such as complement receptor 3 (CR3, αM β2-integrin, CD11b/CD18), lactosylceramide, glycosphingolipid, scavenger receptors, dectin-1, TLR-2, and TLR-4 (Brown *et al.* 2007; Li *et al.* 2011; Moradali *et al.* 2007).

In sum, a new class of antitumor and immunomodulating medicinal mushroom drugs (the biological response modifiers (BRMs)) is emerging in the clinical scene. The application of BRMs as a special type of immunotherapy to target and eliminate cancer cells could represent a new kind of cancer treatment together with surgery, chemotherapy, and radiotherapy (Mizuno 1999; Wasser 2002, 2014). Findings suggest that some mushrooms work in synergy with commercial anticancer drugs as an effective tool for treating drug-resistant cancers. Antitumor monoclonal antibodies in conjunction with β-glucans have been considered as a novel anticancer immunotherapy against GD2 ganglioside, G250 protein, and CD20 protein, respectively in experimental neuroblastoma, carcinoma, and $CD20^{+}$ lymphoma (Vannucci *et al*. 2013; Xiang *et al*. 2012). Mushroom β-glucans might also have synergistic effects with monoclonal antibodies used in cancer treatment similar to yeast β-glucans.

More than 30 mushroom extracts and fungal compounds are currently being investigated in clinical trials by the National Institutes of Health in the US. Table 5.2 lists some of these clinical trials with mushroom polysaccharides or polysaccharide-rich extracts/powders. The addition of new areas of application, apart from the immunological use in oncology, opens interesting perspectives and makes the study of β-D-glucans a prospective field of research. For example, β-D-glucans also appear suitable for use in nanomedicine for preparation of nanocarriers for drug or biological molecule delivery (Soto *et al*. 2012).

Table 5.2 Selection of recent clinical trials conducted with polysaccharide-rich mushroom-derived preparations.

Official title	Intervention	Subjects	Purpose
Immune Benefits from Mushroom Consumption (University of Florida/ Mushroom Council)	Dietary supplement: 3 or 6 ounces (around 28 g) daily for 4 weeks	52 healthy patients	To determine whether consuming shiitake polysaccharide-rich mushroom is effective in enhancing the

Last updated: December 2, 2013			function of γ δ T cells
A Translational Breast Cancer Prevention Trial of Mushroom Powder in Postmenopausal Breast Cancer Survivors (City of Hope Medical Center/ National Cancer Institute) (NCI) Last updated: June 5, 2014	Drug: white button mushroom extract Dose escalation beginning at 5 g/day, then 8, 10 up to 13 g/ day	16 females with breast cancer, 21 years and older	To show that a whole food extract of white button mushrooms can inhibit aromatase-induced estrogen biosynthesis in women who are breast cancer survivors
Does Maitake Mushroom Extract Enhance Hematopoiesis in Myelodysplastic Patients? A Phase II Trial (Memorial Sloan-Kettering Cancer Center/ Yukiguni Company)	Patients will receive maitake mushroom extract orally 3 mg/kg twice daily for 3 months	43 myelodysplastic patients, age 18 or older	To see whether maitake improves the hematopoietic response, in particular, neutrophil count and function, in myelodysplastic patients

Last updated: September 3, 2014			
Efficacy and Safety of Cauliflower Mushroom Extract on Promotion of Immunity (Chonbuk National University Hospital) Last updated: November 26, 2012	Phase II and III Dietary supplement: cauliflower mushroom extract (1 g/ day), for 12 weeks	60 males and females, 30 years to 65 years	To evaluate the efficacy and safety of cauliflower mushroom extract on promotion of immunity (IL-10, IFN- γ, TNF-α, and blood cell counts)
Phase Ib of Mushroom Powder in Biochemically Recurrent Prostate Cancer (City of Hope Medical Center/ National Cancer Institute) (NCI) Last updated: October 9, 2014	Drug: white button mushroom extract. Dosages: 4, 6, 8, 10, 12, and 14 g/day	36 male patients	To study the side-effects and best dose of white button mushroom extract in treating patients with recurrent prostate cancer after local therapy
A Randomized, Parallel,	Dietary supplement: Yunzhi extract	60 women patients with diagnosis of	To assess the effects of the traditional

Double-blind, Placebo-controlled, Pilot Clinical Study on the Effects of Yunzhi as Dietary Supplement in 60 Adult Patients Undergoing Adjuvant/ Neoadjuvant Chemotherapy for Breast Cancer (Hospital Clinic of Barcelona) Last updated: December 14, 2010	from *Coriolus versicolor* 3.5 g/day	breast cancer, 18 years and older	Yunzhi mushroom, as adjuvant in the treatment of patients with breast cancer
Use of the Medicinal Mushroom *Agaricus blazei* Murill in Addition to High Dose Chemotherapy in Patients With Multiple Myeloma (Ullevaal University Hospital)	Phase II Intake of 60 mL *A. blazei* daily in addition to chemotherapy. Commercial name: AndoSan™	39 patients scheduled to undergo high-dose chemotherapy with autologous stem cell support for multiple myeloma	To assess the effects of *Agaricus* extract (AndoSan™) in addition to chemotherapy on cytokine levels as well as treatment response and quality of life of patients with multiple

Last updated: February 22, 2014	myeloma

Adapted from: http://clinicaltrials.gov/ct2/results?term=mushroom

In addition to high molecular weight polysaccharides, another anticipated application of mushroom species is concerned with the active pool of secondary metabolites with low molecular weight (phenolic acids, flavonoids, terpenoids, lactones, quinones, steroids, and alkaloids) that have antitumor, antimicrobial, and antiviral properties. The scientific investigation of these compounds has gained momentum in recent years because they are simpler chemically and equivalent to existing fungal-based pharmaceuticals, such as penicillin and cephalosporins (Patel & Goyal 2012; Paterson & Lima 2014).

Mushroom terpenoids (tri- and sesquiterpenes) have cytotoxic, antibacterial, antifungal, hypocholesterolemic, hypoglycemic, hypotensive, and antioxidant effects (Badalyan 2014). About 400 bioactive molecules have been isolated from *Ganoderma* species: *G. lucidum*, *G. applanatum* (Pers.) Pat., and *G. tsugae* Murrill. Among them, lanostane-type triterpenoids are promising candidates for the development of antitumor drugs (Fatmawati *et al*. 2013). Ganoderic acids, ganoderenic acids, ganodermic acids, applanoxidic acids, ganoderals, ganoderols, lucidone, ganodermanontriol, and ganodermanondiol are some of the basidiomycetous triterpenoids. In spite of the fact that many triterpenoids have been discovered in mushrooms, few studies have been done to elucidate the mode of action of their anticancer and immunomodulating effects. The research performed on *G. lucidum* has shown that such triterpenoids could activate the NF-kB pathway and modulate Ras/Erk, c-myc, CREB protein, and mitogen-activated protein kinases, leading to other immune activations against tumor cells (Calviño *et al*. 2010; Moradali *et al*. 2007; Petrova *et al*. 2008).

Hispolon, an active phenolic compound extracted from *Phellinus* spp., is known to possess potent antineoplastic properties and to potentiate the cytotoxicity of chemotherapeutic agents. Hispolon induces epidermoid and gastric cancer cell apoptosis and,

regardless of p53 status, it inhibited breast and bladder cancer cell growth. Aa crucial role of hispolon in ubiquitination and downregulation of MDM2 (the protooncogene inhibiting the tumor suppressor function of p53) was reported, suggesting this phenolic compound as an attractive therapeutic strategy in breast, gastric, and bladder cancers (Chen *et al*. 2008; Lu *et al*. 2009).

As for low molecular weight mushroom compounds, only a minute fraction have proceeded to a higher level of clinical evaluation. In this group, irofulven (6-hydroxymethylacylfulvene), a novel synthetic antitumor agent derived from the sesquiterpene illudin S of *Omphalotus olearius* (DC.) Singer, has been one of the most extensively studied. Phase II clinical trials were performed in different tumors (advanced melanoma, advanced renal cell carcinoma, metastatic colorectal cancer, and recurrent or persistent endometrial carcinoma), but unfortunately irofulven demonstrated minimal to no significant antitumor activity in these trials (Zaidman *et al*. 2005). There are still ongoing phase II clinical trials by MGI Pharma in recurrent ovarian cancer, hormone-refractory prostate cancer, and recurrent malignant glioma (http://adisinsight.springer.com/drugs/800006987; Sborov *et al*. 2015).

As mentioned above, low molecular weight mushroom metabolites exhibit an extraordinary diversity but their investigation in clinical trials and use as drugs is currently scarce. Table 5.3 presents an overview of some compounds whose pharmacological activities have been tested at the preclinical level, in some cases with contradictory results depending on the model used, sample concentration, etc. Overall, *in vivo* activity studies are limited when compared with *in vitro* studies. The compound quantity of natural products might be one reason for screening biological activities *in vivo*. Efforts should be made to find new sources for anticancer drugs using low molecular weight mushroom metabolites that can inhibit or trigger specific responses, i.e. activating or inhibiting NF-κB, inhibiting protein and especially tyrosine kinases, aromatase and sulfatase, matrix metalloproteinases, cyclo-oxygenases, DNA topoisomerases and DNA polymerase, antiangiogenic substances, etc. (Chang & Wasser 2012; Patel & Goyal 2012; Petrova *et al*. 2008; Zaidman *et al*. 2005).

Table 5.3 Overview of the pharmacological activity of some low molecular weight compounds from mushrooms in various *in vitro/in vivo* systems.

Sources: Zaidman *et al*. (2005)[1]; Petrova *et al*. (2008)[2]; Calviño *et al*. (2011)[3]; Chang & Wasser (2012)[4]; Patel & Goyal (2012)[5]; Roupas *et al*. (2012)[6]; Öztürk *et al*. (2014)[7]; Paterson & Lima (2014).[8]

Mycochemical family	**Examples of compounds**	**Mushroom**	**Pharmacological effect**
Terpenoids	Irofulven (illudin's derivative)[1,6,8]	*Omphalotus illudens Suillus placidus*	Antitumor activity against human pancreatic carcinoma cell lines *in vitro* and *in vivo*, HT-29 and HCT-116 colorectal and A2780 ovarian carcinoma cells, head and neck, nonsmall cell lung, malignant glioma, colon, ovary and prostate cancer. Phase II clinical trials are ongoing
	Triterpene-enriched fraction WEES-G6 (especially ganoderic acid	*Ganoderma lucidum*	Selective growth inhibition of Huh-7 human hepatoma cells. It caused

	F)[1,7]		a rapid decrease of PKC and the activation of JNK and p38 MAPK protein kinase signaling pathways. Inhibition of angiogenesis in an *in vivo* model
	7-oxo-ganoderic acid Z and 15-hydroxy-ganoderic acid S	–	Inhibition activity against HMG-CoA reductase and acyl CoA acyltransferase.
	Ganoderic acid C2[1,3,7]	–	Apoptosis induction in NB4 human leukemia cells. Effective against cell proliferation and colony formation in MCF-7 human breast adenocarcinoma cell line; mediated G1 cell cycle arrest
	Ganoderic acid X[1,7]	–	Inhibition of DNA synthesis

			in human hepatoma cell lines (Huh-7), inhibition of topoisomerase I and IIα, activation of apoptosis and inhibition of protein kinases
	Lucidenic acid B[1, 7]	–	Implicated in the inhibition of Erk on HepG-2 human liver cells, apoptosis
	Tricholomalides A, B and C[1, 7]	*Tricholoma* spp.	Induction of neurite outgrowth in rat PC-12 cells
	Sarcodonin G[7]	*Sarcodon scabrosus*	Suppression of inflammation induced by TPA, activation of caspases-3 and -9 and increased Bax/Bcl-2 ratio, antiproliferative activity against HOC-21, HEC-1, U251-SP, MM-1CB, and HMV-1 human cancer cell lines.

	Eryngiolide A[7]	*Pleurotus eryngii*	Cytotoxic effects against Hela and HepG2 tumor lines by using MTT assay
Steroids	Ergosterol and ergosterol peroxide[6-8]	Multiple species	Increase serum 25(OH) vitamin D_2 levels (*in vivo* – humans). Antibreast cancer. Direct inhibition of angiogenesis induced by solid tumors. Inhibition of leukemic cells proliferation
	Blazein[6,8]	*Agaricus blazei*	Induction of apoptotic chromatin condensation in human lung cancer cells and stomach cancer cells
	Ergosta-4,6,8(14),22-tetraen-3-one (ergone)[5]	*Russula cyanoxantha*	Cytotoxic and antiproliferative activity towards HepG2 cells through apoptosis induction and G2/M cell

			cycle arrest
Nucleotide-derivatives	Cordycepin[2,4-6,8]	*Cordyceps militaris*	Inhibition of human leukemia cell growth by inducing apoptosis through a signaling cascade involving a ROS-mediated caspase pathway. It continues to be a potentially useful tool to identify therapeutic targets
	Clitocine[6.8]	*Leucopaxillus giganteus*	It targets Mcl-1 to induce drug-resistant human cancer cell apoptosis *in vitro* and tumor growth inhibition *in vivo*
Phenolic compounds	Hispolon[5,7]	*Phellinus* spp.	Inhibition of breast and bladder cancer cell growth, potentiation of cytotoxicity of chemotherapeutic agents used in

			the clinical management of gastric cancer
	Caffeic acid phenethyl ester (CAPE)[1,2,4]	*Agaricus bisporus, Lentinus edodes, Phellinus linteus, Marasmius oreades*	Specific cytotoxicity against tumor cells, shows NF-κB inhibitor activity, and can be a candidate for antitumor drugs, especially against breast cancer
	Genistein (an isoflavone)[1]	*Flammulina velutipes*	Modulates G2/M checkpoint and apoptosis induction and suppresses proliferation of p53 null human prostate carcinoma cells. Inhibition of several tyrosine kinases and topoisomerases. Also acts as antioxidant
Alkaloids	Norsesquiterpene alkaloid[7]	*Flammulina velutipes*	Cytotoxicity against human

			cervical carcinoma KB cells *in vitro* by using the MTT assay
	Isohericenone, isohericerin and erinacerin A[6-8]	*Hericium erinaceum*	Cytotoxic activity against HCT-15, SK-MEL-2, SK-OV-3, and A549
	Sinensine[7]	*Ganoderma sinense*	Biological activity in protecting H_2O_2 oxidation-induced injury on human umbilical vein endothelial cells
Lactones	Clavilactones A, B and D (respectively CA, CB and CD) and two semisynthetic derivatives (diacetyl-CA and dimethyl-CA)[1]	*Clitocybe clavipes*	Inhibitory activity in kinase assays against the Ret/ptc1 and epidermal growth factor receptor (EGF-R) tyrosine kinases, weak inhibition activity when administered to mice bearing the ascitic A431

tumor

MAPK, mitogen-activated protein kinase; MTT, (3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide; NF-κB, nuclear factor κB; PKC, protein kinase C; ROS, reactive oxygen species; TPA, 12-O-tetradecanoylphorbol 13-acetate.

The available information about bioactive molecules of medicinal mushrooms suggests that these may be powerful sources from which to develop novel pharmaceutical products. It is hoped that as technology advances for the production of mushroom drugs, there will be increased clinical research to ensure their safety and efficacy, thus validating many claims made for the medicinal use of these products. As Chang and Miles (2004) stated, “Anecdotal accounts are interesting and may be useful, but scientific experimentation is essential.”

5.4 Conclusion

There is no better time for mushroom products to emerge as judged by their positive impact on human quality of life. Recent basic and applied studies in mushroom metabolism, biotechnology, and clinical trials represent a large contribution to the expansion of mushroom potentialities for the development of functional foods, nutraceuticals, and novel drugs.

Mushroom functional foods represent an opportunity to obtain innovative products that would help to satisfy the demand that already exists. In addition, different mushroom formulations provide health-enhancing nutraceuticals for healthy and subhealthy people. Although not “magic” products like those of “Alice in Wonderland,” based on the multiple biological properties of mushroom nutraceuticals, the view of Stephen DeFelice that “One good nutraceutical can wipe out the drugs” has gained momentum in recent years.

However, many of the bioactive properties attributed to mushroom functional foods and nutraceuticals are based on data obtained from *in vitro* and animal experiments (Vikineswary & Chang 2013). Well-designed and -conducted clinical trials and better insight into the mechanism underlying the biological action of

mushrooms will accelerate commercial production of myco-pharmaceuticals. A more detailed chemical and biological characterization of both high and low molecular weight biologically active compounds from different mushroom species appears necessary to better define the rationale for their application in anticancer therapies as well as in other pathologies. Glucan and proteoglycan immunoceuticals acting as biological response modifiers are effective immune boosters for individuals afflicted with cancer or impaired immunity and possess a unique clinical versatility. Interest in the investigation of new and powerful low molecular weight compounds has increased due to the wide range of their medicinal activities.

The target for the future should be to adopt regulations, standards, and practices from Western and Eastern medicine that have proven to be the most valuable in the quest for health benefits (Wasser 2014). Further sustainable research on the natural and genetic resources of edible and medicinal mushrooms using improved screening methods of OMICs sciences will assist future usage of their bioactive myco-compounds to develop unique health biotech products with a positive impact on human welfare. In sum, this chapter provides insights into the possible uses of mushrooms as functional foods, nutraceuticals, and drugs. The present status and future prospects suggest great potential for upgrading mushroom species from functional food to translational mushroom medicine.

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6
The Consumption of Wild Edible Plants

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6.1 Wild Edible Plants

The long history of humans' ability to adapt to natural environments and to interact with nature and social circumstances is profoundly attached to edible wild plants and animals. From the early hunter-gatherers and across different adaptation stages, plants have assumed great importance in human societies and many people all over the world have depended on many wild species particularly for food and medicines. Specific relations between dietary and therapeutic purposes are intrinsic to wild edible plant use and have been well documented by several researchers (Abbasi *et al*. 2013a; Alarcón *et al*. 2015; Etkin 2008; Etkin & Ross 1991; Grivetti 2006; Ogle *et al*. 2003; Sánchez-Mata *et al*. 2012; Touwaide & Appetiti 2015).

Wild edibles, a term used to describe both plants and animals consumed by humans, can be a rather ambiguous concept as in most cases the simple act of foraging and gathering implies some management of resources and habitats, as observed by Turner *et al*. (2011) and Sõukand and Kalle (2015).

It is generally accepted that wild plant species grow spontaneously in self-maintaining populations in natural or seminatural habitats, existing independently of direct human action (Maurer & Schueckler 1999). They are available in various ecosystems and agroecosystems, with unique significance those related to forests

and trees which play or have played crucial roles in many food systems, providing direct and indirect resources for human nutrition (Vinceti *et al*. 2013). Ruderal species that colonize disturbed sites and weeds (high competitive species from arable field and crop contexts) are also important sources of food (Bye 1981; Maroyi 2013; Turner *et al*. 2011).

Wild edibles include a rich variety of plant lifeforms and botanical features, including annual and perennial herbs, forbs, vines, sedges and rushes, grasses, broadleaved and needle-like or scale-like leaved shrubs, trees, and ferns. Other living organisms have also been considered as plants, e.g. mushrooms, algae, and lichens. On a seasonal basis, roots, underground storage organs, shoots, stems, sprouts, leaves, flowers, fruits and cones, seeds and nuts, bark, galls, nectar and gum, along with fronds, lichens and algae have been included in sustenance obtained from edible wild plant species, *sensu lato*.

In many cases, people have quite different food choices although they live in similar environments and explore identical landscapes. Turner *et al*. (2011) argue that such unequal choices and food patterns are not completely explained by levels of biodiversity, territorial differences or resources availability, but can be due to necessity or opportunity, or to remarkable significance within each human group.

Natural dispersal of plants and human transport of propagules and seeds from one place to another led to a huge number of wild and weed plants that have been traditionally collected and consumed throughout the world. These plants may be either native or exotic species, the latter intentionally or accidentally introduced during the dispersal process and becoming adapted to new habitats (i.e. naturalized).

Distinguishings between wild and cultivated plants is not always an easy task because there are many intermediate stages. Some species growing wild may be cultivated in specific sites and cultivated ones may be naturalized or maintained as semidomesticated. For instance, timber trees are also used for their fruits, e.g. hazel (*Corylus avellana* L.) and walnut (*Juglans regia* L.), in some European regions.

Since most wild plants have never been cultivated, their biodiversity, chorology, biology, and agronomy have remained poorly studied. Plant use and management rely on knowledge and skills developed for centuries on a local scale. This local knowledge (LK), sometimes also known as traditional ecological knowledge (TEK), implying the sustainable use of native resources, relates to adaptive complex systems that include perceptions, beliefs, and practices transmitted through generations. Therefore, the true diversity of wild edibles used is still unknown in many regions and linked ethnobotanical and indigenous/local knowledge is not properly documented.

Existing knowledge of plants and their uses, particularly food uses, is an immense valuable legacy of which some part is being lost every day, creating an enormous urgency for further studies in order to make these resources available for future generations and for food sovereignty and security.

6.1.1 Contribution of Wild Edible Plants to People's Diets and Daily Lives

Ethnobotanical surveys show that more than 7000 species of wild plants have been used for human food at some period throughout human history, having a prominent role in both early and contemporary societies. Grivetti and Ogle (2000) observed that edible wild plants were regular components of the diets of millions of people. Despite the fact that in more recent times human diets have used relatively few plant species, which also encompasses the decline of plant use knowledge, subglobal assessments show that several indigenous and traditional communities currently consume 200 or more species (Grivetti & Ogle 2000; MEA 2005).

For many years, scientists have reported the relevance of wild plants used as either vegetables or medicine. Several research approaches have confirmed that many edible wild plants have therapeutic value in addition to their nutritional importance, due to the presence of biologically active compounds, and thus they can be considered as food-medicine or functional foods (Local Food-Nutraceutical Consortium 2005; Vanzani *et al.* 2011). However, as Etkin and Ross (1982) emphasized three decades ago, nowadays

our understanding of “the added benefit of regular dietary intakes, in low concentrations, of wild plants with known phytochemical properties is still limited” (Etkin & Ross 1982).

More recent studies carried out in different areas (Bharucha & Pretty 2010; Dansi *et al*. 2008; Delang 2007; Ju *et al*. 2013; Łuczaj *et al*. 2013a; Mattalia *et al*. 2013; Quave & Pieroni 2015; Scarpa 2009) show that many people worldwide still rely on local environmental resources, especially wild plants, for daily subsistence and healthcare.

Dansi *et al*. (2008), studying traditional leafy vegetable usage in the Benin Republic (Africa), reported that most of these plant uses have been neglected by scientific research and development agencies, leading to a decline in consumption and diversity. These findings can certainly be generalized to other developing regions, causing, a significant impact on income and the nutritional status of households throughout the entire world.

Dounias and Froment (2011) established how the history of mankind, shifting from a nomadic hunter-gatherer existence to a farming sedentary lifestyle, is marked by a series of major physiological, demographic, cultural, and dietary transitions that are negatively correlated with food security, nutrition, and health. Moreover, based on case studies conducted in Asia (Borneo) and Africa (Cameroon), they note that diets and illnesses are complex indicators of the ecological and cultural costs that indigenous communities currently pay to benefit from modernity (Dounias & Froment 2011).

According to FAO *et al*. (2015), an unacceptably large number of people in the world still lack the food they need for an active and healthy life. The latest available estimates indicate that one in nine people are/will be undernourished in 2014–16 (about 795 million) which is linked to reduced conditions of health and sanitation, inappropriate care, and poor nutritional status. Although this represents a reduction of 21.4% in the last two decades, advancements towards improved food security and safety (Hanning *et al*. 2012) are still not similar across all regions, and undernourishment is greater in many developing ones (e.g. Central Africa and Western Asia).

Shortage of food is particularly high in many rural areas where family farming systems and smallholder agriculture are predominant. Such agricultural schemes are recognized as playing key roles in reducing hunger and poverty (FAO *et al*. 2015). Globally, they are characterized by intense relationships with nature, important crop diversity, and particular resources management to avoid productive risks and encompass wild resources and relevant LK or TEK. In addition, these agricultural heritage systems have relied on generations of family farmers, considered custodians of biodiversity, for their contribution to the preservation of traditional food products, safeguarding the world's agrobiodiversity and sustainable use of natural resources (FAO 2014).

In many communities, lacking basic infrastructure and market access, wild gathering provides considerable subsistence support to local diets (Stryamets *et al*. 2015; Sunderland 2011) and may also generate further benefits (e.g. selling surpluses) (Delang 2006). Nevertheless, Sunderland (2011) notes that gathering from the wild or growing food (family farming and smallholder agriculture) are not enough to meet nutritional needs in developing regions; accordingly, the most vulnerable peoples are particularly at risk of privation and lack of access to food. The report on the state of food insecurity in the world (FAO *et al*. 2015) expresses that "progress towards food security and nutrition targets requires that food is available, accessible and of sufficient quantity and quality to ensure good nutritional outcomes."

6.1.1.1 Famine Foods

The ethnobotanical literature emphasizes the importance of wild edibles under conditions of food shortage, crop failure and seasonal variations, diseases, climatic adversity, and social or political conflicts (Bvenura & Afolayan 2015; Delang 2006; Grivetti 2006; Kang *et al*. 2012; Nascimento *et al*. 2012; Panda 2014; Scarpa 2009; Stryamets *et al*. 2015). Some species have potential dietary use but are not regularly eaten during normal periods.

Wild and forest foods play a significant role as a source of

resilience in the food system. Several surveys have reported how different communities worldwide are able to manage plant resources when food insecurity is highest, specifically during dry or wet seasons according to different climatic regions (Grivetti 2006; Powell *et al*. 2014; Somnasang & Moreno-Black 2000; Svanberg 2012).

For all these reasons, many wild edible plants are seen as something linked to poverty and nutritional deficits, in addition to precarious livelihoods. Frequently, lower incomes are insufficient to buy commercial food crops and staples, which are perceived as signs of progress, modernity, and higher status (Delang 2006).

Lack of knowledge and inability to identify plants existing in the wild and available to sustain survival have led to malnutrition and hunger in certain areas of the world (Grivetti & Ogle 2000).

Some examples of critical foods from the reviewed literature are the corms from *Colchicum montanum* L. used in the Mediterranean region (Leonti *et al*. 2006); the Brassicaceae, wild mustard, *Sinapis arvensis* L., and wild radish, *Raphanus raphanistrum* L. used in Poland (Łuczaj 2010); the starchy rhizomes of waterlilies from the family Nymphaeaceae used by Native Americans and Australian Aborigines, and the inner bark of some gymnosperms in north-western North America (Turner *et al*. 2011); the leaves of *Glechoma hederaceae* L. used for seasoning broths and soup in north-eastern Portugal (Carvalho & Morales 2013).

6.1.1.2 Weeds

Grivetti and Ogle (2000) highlight the importance of edible weeds within regional food security, referring to the concept of *hidden harvest*. Weed species are closely related to crops and agricultural farming systems, and are of nutritional relevance, as reported by several authors (Bye 1981; Maroyi 2013; Molina *et al*. 2014).

Food uses of most of these species comprise the ingestion of raw immature herbaceous leaves and stems although for some the edible portion corresponds to bulbous leaf bases.

Weeds from arable crops and disturbed environments are consumed in several African and Asian countries mainly as vegetables, according to a brief review by Maroyi (2013). Likewise, the author found in other studies that weeds used as traditional greens in Zimbabwe are frequently undervalued by research and governmental institutions, although they are an important part of daily food intake, supplementing conventional vegetables and some being preserved for later use.

Molina *et al.* (2014) evaluated the potential sustainable exploitation of weed vegetables traditionally consumed in the Mediterranean region, which are known to be rich in bioactive compounds that might have important health benefits because of their antioxidant activity. The authors were able to provide quantitative data on yield and availability of 15 Mediterranean wild green vegetables. Edible yields of the studied species were found to be high in most cases, confirming their potential to increase food diversity. Some of the most appreciated of the local wild gathered species, such as *Scolymus hispanicus* L. (Asteraceae) and *Silene vulgaris* (Moench) Garcke. (Caryophyllaceae), showed low production rates, which suggests that yield and availability are not the main criteria for local selection of wild edible species (Molina *et al* 2014).

It is worth noting that weeds occur in marginal lands, are easily accessible in quantity and, in general, are at low risk of overexploitation. These plants are some of the neglected and underutilized wild species that have associated potential benefits, in terms of nutritional relevance, food security, medicinal value, income generation, economic growth, and cultural advantages.

6.1.2 New Trends in Edible Wild Plant Consumption

Another aspect of wild edibles consumption is the latest trends (Łuczaj *et al.* 2012) based on local traditional behaviors. In many European countries (Dénes *et al.* 2012; Kalle & Sõukand 2012; Łuczaj 2012; Łuczaj *et al.* 2013a,b; Molina *et al.* 2014;Redžić 2006; Tardío 2013; Tardío *et al.* 2006), including rural communities of the Mediterranean (Biscotti & Pieroni 2015;

Leonti *et al.* 2006), wild gathered species play a vital role in supplying seasonal food and weed greens and are considered most relevant in terms of nutrition and health (Morales *et al.* 2014; Vanzani *et al.* 2011) and as signs of the cultural identity of such regions. Moreover, they are seen as appealing gastronomic resources for modern culinary experiences. Many restaurants include wild gathered ingredients on their menus and rely far more on home-grown, farmed, and forage foods.

Reyes-García *et al.* (2015) surveyed seven sites in the Iberian peninsula and one in the Balearic Islands in order to identify current trends in the consumption and gathering of wild edible plants. Using information from interviews, they found a generalized decrease in the consumption and gathering of wild edible plants, but while some uses are being abandoned, others remain relatively popular. They conclude that local gastronomic traditions, high cultural appreciation, and recreational functions may explain these tendencies. Currently, the role of wild edible plants as provisioning services is marginal and cultural ecosystem services and nonfood use values may justify the persistence of some uses.

Nowadays, wild edible plant foods serve commercial and recreational purposes too and have a renewed meaning for many rural areas. In some European countries and in Morocco, commonly consumed species of wild edibles, particularly herbs, greens, and berries, are available in local markets (Carvalho 2010; Łuczaj *et al.* 2013a; Powell *et al.* 2014; Svanberg 2012) where they may be bought by inhabitants and by foreigners visiting the area. Small businesses and industries for processing wild edibles, for example marmalades and preserves, are common in some rural areas, such as north-eastern Portugal. Agritourism in Europe is a developing activity gaining popularity; it is highly related to contact with countryside and sustainable wild gathering. Several outdoor initiatives also promote wild edible foods as a recreational activity (Stryamets *et al.* 2015; Svanberg 2012); collecting and consuming such species are much appreciated and provide important cultural ecosystem services, comprising cultural landscape, recreation, and identity (Schulp *et al.* 2014). Surviving in the wild is a new approach in more economically developed

societies. Users searching the web can easily find different field guides for subsisting on wild edibles from Europe, North America, Canada, and Australia. An example of wilderness survival using wild plants as food is mentioned by Svanberg (2012) in Sweden.

A case study focusing on incentives for wild plant gathering shows that, at least in Europe, there is a growing interest in such activity, after being abandoned over the last decades (Schunko *et al*. 2015). Although their outcomes cannot be generalized, the authors have identified five types of motivation for gatherers (quality type, fun type, traditional type, income-oriented type, and nongatherer type). Gathering from the wild has gained popularity and fashionable attention because people prefer quality products with known provenance, and enjoy direct contact with nature and the activity itself. So for many, the motivation for wild food collection has changed from the necessity of satisfying diverse essential needs to the preference for quality products and pleasure of collecting. These motivations denote a positive self-perception and personal commitment to plant gathering from the wild, enabling persistence of plant knowledge and wild gathering specifically (Schunko *et al*. 2015).

Global movements, such as the Slow Food and Terra Madre networks, were founded to prevent the disappearance of local food cultures and traditions, contributing to raising awareness about food security, perceived as quality, variety and access to food, with a commitment to consumers, producers, cultural diversity, and the environment (www.slowfood.com).

These new attitudes also represent changed perceptions about wild plant gathering and consumption. As mentioned before, until very recently, many cultures harbored a prejudice against wild edibles, leading to a decline of interest; such foods were negatively associated with starvation (Carvalho & Morales 2013) and considered “famine foods” (Kang *et al*. 2012; Nascimento *et al*. 2012). However, negative insights and attitudes towards wild foods are still reported in many studies conducted in Africa (Bvenura & Afolayan 2015) and Asia (Panda 2014), where wild edibles are literally considered as “nourishment for women, children and the weak,” natural disasters foods (e.g. flood or drought), and tasteless and unappetizing but necessary resources

during acute food shortage (Addis *et al.* 2013).

6.1.3 Wild Edible Plants, Food Security, and Research Approaches

Multidisciplinary studies of wild edible resources need to be conducted because it is already evident that local ecological knowledge about traditional and particular diets will benefit humankind in many ways; however, this heritage is largely decreasing due to economic, ecological, and societal changes. Food security, safety and sovereignty, subsistence, undernourishment, and new ideas about food and health are two sides of the same coin.

Sustainable diets are deeply interconnected with several key factors such as food and nutrients needs, wellbeing and health, food security and accessibility, seasonal foods, equity and fair trade, biodiversity and environment, local development, traditional knowledge and skills, and cultural heritage (Lairon 2012).

Combining traditional knowledge and expertise with more recent concepts and applied research is a useful approach but public policies, increasing human rights to food, health, and welfare, in addition to enhancing biodiversity and ecosystems services, are also required. Appropriate transdisciplinary abilities and attitudes are needed to improve staple foods yields in a sustainable way, while protecting natural and crop biodiversity, as well as avoiding harmful anthropogenic effects on the biophysical environment (de Schutter 2011).

6.2 Foraging and Wild Edible Plant Resources

Foraging, the act of searching for food or provisions, was a form of social organization with profound implications in many cultures. Foraging and wild gathering embody a deep knowledge

of plants and sites, sustainable practices of handling the available resources, daily interaction with nature and environment, and the answer to limited food supplies.

Bharucha and Pretty (2010) undertook a detailed analysis of the best existing evidence for the roles and values of wild foods and their relation to agricultural systems. They found that, for many reasons, foraging and gathering should not be considered outdated and an earlier stage of human evolution, but just a way to adapt to different ecological and socioeconomic circumstances. They also suggest that foraging and farming practices overlap, and people manage and improve wild and agricultural resources in the same manner using similar approaches and techniques; both activities are thus complementary.

In many cultures, a multitude of wild edible plants were, and sometimes still are, included in the food basket, contributing to macro- and micronutrient intake. Many of these species are versatile and quite often, women's knowledge and skills are fundamental for using and managing wild edibles. Besides providing food and medicine, these plants may be traded and generate cash income. Opposing forces and attitudes influence decisions on plant use and wild gathering practices, endangering the reservoir of diversity available for conservation of traditional foods and for a broad understanding of the role of wild plants in health and nutrition.

Increased demands from a growing population, the rapid expansion of intensive agriculture, the loss of forest cover and changes in essential habitats, greater pressure on ecosystems and biodiversity, and the lack of sustainable use linked to LK or TEK are the principal factors threatening wild plant resources and are absolutely critical to its accessibility (MEA 2005).

6.2.1 Wild Plant Resources Worldwide

Although there is rising interest in developed societies, there are also clear signs of an accelerated decline in wild species use and associated local knowledge and management practices. However, wild edibles are still consumed across both industrialized and developing countries.

Turner *et al*. (2011) produced the most comprehensive review to date of various categories of edible wild and tended plants used in different regions of the world, and they discuss the concept of tending and managing not only wild plants but fungi and algae as well. They also emphasize the richness and diversity of wild food and its contribution to nutrition and cultural identity, reflecting important TEK.

The different kinds of edible parts obtained from wild species are commonly consumed in different ways according to particular cultures and specific needs. Moreover, recent scientific approaches (see Chapters 7 and 8) have confirmed the nutritional value of many of these foods. For instance, numerous fruits and seeds have useful vitamin content and appreciable amounts of soluble fiber and antioxidant compounds such as ascorbic acid (Barros *et al*. 2010, 2011a; Morales *et al*. 2013); many sprouts, stems, leaves, and aerial parts are rich in micronutrients (Martins *et al*. 2011; Morales *et al*. 2014; Pereira *et al*. 2011; Sánchez-Mata *et al*. 2012); some underground organs (roots, tubers, corms, bulbs, and rhizomes) and tropical fleshy fruits have rich starchy cells and pulp and a high fat content, contributing to human caloric needs (Crowe 2005; Hladik *et al*. 1984; Kuhnlein & Turner 2009).

Furthermore, in the Mediterranean region seasoning is a very important practice, primarily for the taste and aroma it imparts to food but also for the nutritional value of the main species consumed (Barros *et al*. 2011b; Pardo de Santayana *et al*. 2007), as well as for its role in preservating sauces, sausages, meat, and fish (Póvoa *et al*. 2006). Asian gastronomy also uses a strong aromatic component; herbs, leaves, and seeds of wild species are key ingredients used to make vegetal oils or flavor food (Bortolotto *et al*. 2015; Ju *et al*. 2013; Li *et al*. 2015; Rajasab & Isaq 2004). All over the world, leaves, flowers, and fruits of wild native plants have been used for flavor (steeping in water) and to prepare beverages (fermenting and distilling) that were used in rituals or events with cultural and religious significance (Bortolloto *et al*. 2015; Estrada-Castillón *et al*. 2014; Hong *et al*. 2015; Kuhnlein *et al*. 2009; Pardo de Santayana *et al*. 2007; Sõukand *et al*. 2013).

Some wild edibles may be eaten fresh and raw, such as greens and

fruits; others require previous preparation (e.g. peeling or deseeding). Plus, for many, further procedures are needed to render them digestible or to remove toxins and poisonous constituents (e.g. destemming, blanching, leaching or boiling; see Chapter 7). For storage and preserving purposes, several practices are used: dehydrating by sun, wind or heat; hanging and shade drying at room temperature; steaming or hanging in smoke; burying or storing in specific containers (e.g. baskets and wooden boxes); soaking in water; steeping in olive oil, honey, wine or brandy; preserving in pig fat or other fats and oils; simply mashing with spices, garlic and vegetal oils or animal greases; combining previous roasting with mashing and seasoning; making pastes; preserving in vinegar or salt water; baking or processing in jams, jellies, and conserves; fermenting (Carvalho 2010; Kuhnlein & Turner 2009; Póvoa *et al*. 2006; Quave & Pieroni 2014).

A review of the literature provides relevant information about wild edible plant resources explored within different ecosystems (e.g. tropical and temperate forest, grasslands, wetlands) in many parts of the globe. These works document local knowledge and consumer procedures with reference to indigenous, rural, migrant or urban peoples, and reflect both historical and recent data.

The different contexts, methodological approaches, and tools applied in most of the studies meana that it is impossible to rigorously compare data, but the number of species per area or per inhabitant is less significant than which and how species are used. Likewise, in most cases, it is also practically impossible to estimate intakes or to generalize the described patterns of consumption across different user groups. Therefore, selected examples, compiled from the latest publications found using the keyword *wild edible plants*, give an interesting overview of the range of species and the pattern of plant uses recently documented worldwide (Table 6.1).

Table 6.1 Selected examples of recent literature reporting plant use of wild food species from all over the world. The key words *wild edibles plants* and Google search engine were used to find the last studies. Only Equisitopsida (APG III, 2009), formerly Embriophyta, are considered. Data are organized by descending alphabetical order of region's name and main continents (Africa,

Asia, Europe, Americas).

Region	No. of wild edible species	Habitat	Lifeform	Food category	Botanical diversity/ most reported families	References
Amuria, north-eastern Uganda, East Africa	51	Savannah	47% herbs 39% trees	51% fruits 43% greens	**32 botanical families** Malvaceae (17%) Fabaceae, Moraceae, Solanaceae (13%)	(Ojelel & Kakudidi 2015)
Konso Wereda, South Ethiopia, north-eastern Africa	127	Great Rift Valley, plateaus, wide topographic induced climatic variation	62% shrubs, trees 28% herbs 10% vines	26% greens 12% ground organs 4% fruits	**45 botanical families** Malvaceae (11%) Fabaceae (9%) Apocynaceae (8%)	(Addis *et al.* 2013)
Morocco, northern Africa	246	Mountainous and Sahara areas	66% herbs 34% trees	31% greens 14% seasoning 13% fruits 9% baking	**60 botanical families** Asteraceae (13%) Lamiaceae (8%) Fabaceae (7%) Brassicaceae (5%)	(Nassif & Tanji 2013)
Nhema,	67	Tropical	39%	67%	**30**	(Maroyi

Midlands Province, Zimbabwe, southern Africa		savannahs and scrublands biome, semiintensive farming	trees 31% shrubs 27% herbs	fruits 15% greens	**botanical families** Anacardiaceae (9%) Moraceae (9%)	2011)
Shurugwi District, Midlands Province, Zimbabwe, southern Africa	21	Tropical savannahs and scrublands biome, semiintensive farming	Weeds 55% herbs	81% greens	**11 botanical families** Amaranthaceae (19%) Asteraceae (14%) Malvaceae (14%)	(Maroyi 2013)
Five provinces of South Africa	103	Different physiographic and climate conditions	68% herbs 7% trees 6% vines	95% greens 19% fruits	**33 botanical families** Amaranthaceae (13,5%) Malvaceae (12%) Asteraceae (9%)	(Bvenura & Afolayan 2015)
Sudanian & Sudano-Guinean regions, Benin, west Africa	70	Woodland and savannah	36% trees 33% herbs	57% greens 47% fruits	**38 botanical families** Asteraceae (10%) Anacardiaceae (8%) Bombacaceae (5%)	(Segnon & Achigan-Dako 2014)
Gongba Valley, Gansu, China,	76	Vegetation from desert, through	34% herbs 30% trees	46% fruits 40% greens	**21 botanical families** Rosaceae	(Kang *et al.* 2014)

eastern Asia		dry grasslands, to deciduous forests in the mountainous	25% shrubs		(21%) Asteraceae (9%) Caprifoliaceae (8%)	
Hassan District, Karnataka, India, south Asia	29	Deccan plateau, extreme diversity of climatic conditions, and wide vegetation range	Mainly trees and herbs	66% greens	**19 botanical families** Fabaceae (21%) Poaceae (17%) Arecaceae (10%)	(Prashanth Kumar & Shiddamallaya 2015)
Kendrapara District, India, south Asia	86	Central coastal plain zone, warm and humid climate	38% trees 29% herbs	37% greens 33% fruits	**51 botanical families** Amaranthaceae (7%) Fabaceae (6%)	(Panda 2014)
Kupwara, India, south Asia	28	Himalayan mountains and several valleys, climate monsoonal, diverse biogeography	89% herbs 7% shrubs	79% greens 18% fruits	**17 botanical families** Asteraceae (14%) Amaranthaceae (11%) Polygonaceae (11%)	(Mir 2014)
Nanyi, Milin	27	Extreme altitudinal	41% herbs	65% fruits	**12 botanical**	(Li *et al.* 2015)

County, south Tibet, China, Asia		differences, subtropical humid and semihumid climate, temperate semihumid monsoon forest	34% shrubs 24% trees		**families** Berberidaceae (17%) Lamiaceae (17%) Rosaceae (17%)	
Nepal, south Asia	74	Mainly broadleaf forest and subtropical pine forest	51% trees 19% herbs	54% fruits 44% greens 15% pickles	**39 botanical families** Moraceae (11%) Anacardiaceae (9%) Fabaceae (6%) Euphorbiaceae (5%)	(Uprety *et al.* 2012)
Qinling Mountains, Shaanxi, central China, eastern Asia	185	Mountainous, humid temperate climate, deciduous forest, highly diverse flora	69% herbs 15% shrubs 13% trees	62% greens 21% fruits 12% ground organs	**67 botanical families** Rosaceae (16%) Asteraceae (11%) Brassicaceae (5%)	(Kang *et al.* 2013)
Shangri-La, Yunnan, China, eastern Asia	157	Mountainous, unique geographical location and climate diversity.	43% herbs 30% shrubs	51% greens 49% fruits 11% seasoning 7%	**51 botanical families** Rosaceae (22%) Brassicaceae (6%)	(Ju *et al.* 2013)

		17 000 species of higher plants, 15% endemic		wine	Asparagaceae (6%) Araliaceae (4%)	
Alava, Basque Country, Spain, southern Europe	73	Mountainous Mediterranean and Eurosiberian transition	56% herbs 28% trees 16% shrubs	56% greens 45% fruits 22% seasoning and liqueur	**32 botanical families** Asteraceae (15%) Rosaceae (15%) Lamiaceae (12%)	(Alarcón *et al.* 2015)
Czech Republic, central Europe	150 (175)	Landlocked, oceanic and continental climate, mixed, conifer and broadleaf, forests	74% herbs 12% trees 11% shrubs	49% greens 21% seasoning 17% fruits	**57 botanical families** Asteraceae (13%) Rosaceae (9%) Brassicaceae (7%)	(Simkova & Polesny 2015)
Emilia-Romagna Region, Bologna, northern Italy, Europe	66	Mediterranean edaphic conditions and flora	48% herbs 33% trees 8% shrubs	38% greens 26% liqueurs 21% fruits 20% snacks	**33 botanical families** Rosaceae (21%) Asteraceae (14%) Lamiaceae (11%)	(Sansanelli & Tassoni 2014)
Gargano, Foggia	79	Hilly, Mediterranean	92% herbs	100% greens	**19 botanical**	(Biscotti &

Province, northern Apulia, Italy, Europe		climate and extremely rich flora, ca. 2100 species, many endemic	and forbs 4% vines		**families** Asteraceae (38%) Brassicaceae (13%) Apiaceae (8%)	Pieroni 2015)
Turkey & Balkans, south-eastern Europe	60	Mostly mountainous, climate diversity, European ecoregion, high proportion of endemic species	75% herbs, 11% trees	100% leaves used for preparing *sarma*	**23 botanical families** Polygonaceae (18%) Malvaceae (17%) Asteraceae (13%)	(Dogan *et al.* 2015)
Rio Negro, central Guatemala, Central America	44	Plateaus, subtropical moist and dry forest	43% trees 27% herbs	55% fruits 30% greens	**26 botanical families** Solanaceae (19%) Fabaceae (16%) Amaranthaceae (12%) Arecaceae (12%) Cactaceae(12%)	(Turreira-García *et al.* 2015)
Paraguay River, Pantanal, Brazil, South	54	Floodplain and areas of residual relief,	Mainly trees and shrubs	81% fruits 19% seasoning 7%	**31 botanical families** Arecaceae (26%)	(Bortolotto *et al.* 2015)

America		riparian, deciduous and semideciduous forests, and Cerrado flora	wine	Fabaceae (16%) Myrtaceae (13%)

6.2.1.1 Africa

According to data cited by Bharucha and Pretty (2010), 1500 wild edible plant species were reported for Central and West Africa. Additionally, Maroyi (2014) documented 24 taxa of ferns belonging to 14 genera and 11 families of pteridophytes that are still used in sub-Saharan Africa. During the last decade, many authors have studied wild edibles consumption and related local knowledge in African regions and countries. Some examples are summarized within the following paragraphs.

A total of 140 species of wild leafy vegetables was inventoried within 29 ethnic areas in Benin, West Africa (Dansi *et al*. 2008); three ethnic groups of the Democratic Republic of Congo consumed 166 wild species (Termote *et al*. 2010); in Ethiopia, north-eastern Africa, 413 wild edible plants were compiled from different ethnic groups in three different territories of the Tshopo district (Lulekal *et al*. 2011), and 127 plants were listed in the Konso district (Addis *et al*. 2013); 27 species were used as sources of food and beverage in Botswana (Neudeck *et al*. 2012); in Marmoucha, Middle Atlas, 246 species were reported (Nassif & Tanji 2013); in Benue State, Nigeria, 42 plants (Shomkegh *et al*. 2013); within three provinces of Morocco (Powell *et al*. 2014), 30 species of wild leafy greens; in Obalanga, Amuria District, Uganda (Ojelel & Kakudidi 2015), 51 species were registered; and 103 species were mentioned in five provinces from South Africa (Bvenura & Afolayan 2015).

These findings suggest that there is still a remarkable array of wild

plants with potential use, at least for their nutritional and health value as already confirmed by applied research (Chetty 2013; Omoyeni *et al*. 2015; Schönfeldt & Pretorius 2011). Such species also have an economical role within rural households and small farmers' incomes and in attempting food insecurity alleviation.

Wild leafy vegetables and underground organs (e.g. roots, tubers, and rhizomes) are well known useful foods, being central components of diets in across Africa (Bvenura & Afolayan 2015; Chweya & Eyzaguirre 1999; Dansi *et al*. 2008; Lulekal *et al*. 2011; Nassif & Tanji 2013; Neudeck *et al*. 2012; Powell *et al*. 2014). The wild leafy food category includes plant materials ranging from leaves of annuals and shrubs to leaves of trees of major plant groupings such as angiosperms, but Maroyi (2014) also provided evidence of the importance of pteridophytes as food sources.

Some priority traditional leafy vegetable species used in Botswana, Cameroon, Kenya, Senegal, and Zimbabwe have been identified (Chweya & Eyzaguirre 1999) such as *Amaranthus dubius* Mart. ex Thell., *Brassica juncia* (L.) Czern., *Cleome gynandra* L., *Corchorus olitorius* L., *Hibiscus esculentus* L. and *Hibiscus sabdariffa* L., *Solanum nigrum* L., *Sonchus cornutus* Hochst. ex Oliv. & Hiern.

In many African countries, some wild species are very popular and are grown in home gardens and sold at local markets (e.g. *Cleome gynandra*, *Corchorus olitorius*, and *Amaranthus* spp.). Other noteworthy species are those from the genera *Adansonia*, *Cassia*, and *Dioscorea*.

6.2.1.2 Americas

There are many significant works focusing on the importance of wild edibles throughout the New World, comprising North America and South America and associated islands. This territory encompasses a wide variation in geological, climatic, and ecological conditions, which have influenced landscape, biodiversity, human history and consequently the development of traditional knowledge about useful plants.

Native American people used a very wide range of plant species for food. Some examples are found in works by Lévi-Strauss (1952) about wild plants in tropical South America and by Krochmal *et al*. (1954) focusing on native plants in the American south-western deserts.

Based on preview surveys, Morton (1963) provided a comprehensive list of the main wild food plants of the United States, excluding Alaska and Hawaii. This included about 1500 species and summarized information about plant parts consumed, processing methods, and potential hazards.

An analysis of food and drug plants of Native North America was performed by Moerman (1996). A database was created comprising a total of 44 775 items, describing the use of various plant taxa by Native American groups, representing 291 different tribes and 3895 uses of different species, 3380 of them vascular plants, of which 1625 species and 10 328 items concern food use. Most native groups used 50–150 food species. Liliaceae s.l., Rosaceae, Ericaceae, and Apiaceae are families widely used for foods (Moerman 1996).

A study from southern Ecuador documents 354 species of wild edible plants, belonging to 66 families, mostly consumed raw. Fabaceae (37 spp.), Arecaceae (29 spp.), Solanaceae (28 spp.), Ericaceae and Myrtaceae (each 23 spp.) are relevant families. Most plants inventoried (85%) have edible fruits. Twenty-two species have edible seeds; some are eaten like nuts, raw or roasted (van den Eynden *et al*. 2003).

Ethnic groups of Gran Chaco, Argentina, used a total of 179 native plant taxa belonging to 46 botanical families; 46.5% of the reported species are Cactaceae (27 species) and 11% are from Apocynaceae, Fabaceae, and Solanaceae (19 species each) (Arenas & Scarpa 2007; Scarpa 2009).

The history of California Indian dependency on and knowledge of the natural world and landscape was highlighted by Anderson (2005). All types of lifeforms from the rich local flora and fauna were gathered from below sea level to above the timberline. A great variety of native vascular and nonvascular plants (e.g. mosses, liverworts, and hornworts) was utilized by different tribes for many purposes, such as foods and medicines. Plant materials

provided 60–70% of the primary nourishment in aboriginal California; one tribe relied on nearly 160 plant species for food and more than 110 plant species for medicines. A rich and balanced diet was based on four established food categories; seeds and grains; bulbs, corms, rhizomes, taproots, and tubers; leaves and stems; and fleshy fruits. Seeds of wildflowers and pines, the grains of native grasses, and acorns of oaks were among the staples of most Indian diets (Anderson 2005).

Kuhnlein and Turner (2009) produced the most complete review of plant foods easily accessible online. They documented traditional plant foods of the indigenous peoples of Canada and neighboring areas and found 550 different species of plants *sensu lato* (including algae, fungi, ferns, and lichens) that provide different food categories (e.g. greens, fruits, grains or mushrooms) and, sometimes, more than one type of edible product per species.

Data from interviews conducted in different environments in different parts of Brazil (Amazon Forest, Brazilian savannah, and the south-eastern coast of the Atlantic Forest) are discussed by Hanazaki *et al*. (2006). Most of the species used have edible fruits but usually shoots, roots or leaves are used in folk remedies.

Surveys from Brazilian dry forest (Cruz *et al*. 2014; Nascimento *et al*. 2012) present extensive information on wild food plants known and used by local people. Comparing different areas, these authors analyze the actual patterns of plant use and people's perceptions of food plant resources.

Examples of some edible wild plants from the Americas, chosen randomly, are the tuberous roots of hog peanut, *Amphicarpa bracteata* (L.) Fernald (Fabaceae); the fruits and roots of Brazil plum, *Spondias tuberosa* Arruda (Anacardiaceae); fruits of prickly pear cactus, *Opuntia* sp. pl. (Cactaceae); berries from *Rubus* sp. pl. (Rosaceae) and *Vaccinium* sp. pl. (Ericaceae); leaves of *Stanleya pinnata* (Pursh.) Britton (Brassicaceae); *Passiflora* sp. pl (Passifloraceae); *Agave* sp. pl. and *Yucca* sp. pl (Asparagaceae); and sugar maple, *Acer saccharum* Marshall (Sapindaceae), among many others.

6.2.1.3 Asia

The last five years have been quite prolific in terms of wild food species research within the different Asian regions, providing interesting and significant information about species, distribution, and availability, as well as plant uses and knowledge (Boesi 2014; Chen & Qiu 2012; Ghorbani *et al*. 2012; Hong *et al*. 2015; Ju *et al*. 2013; Kang *et al*. 2012, 2013, 2014; Li *et al*. 2015; Panda 2014; Uprety *et al*. 2012).

There are estimated to be 1000–2000 edible wild plant species existing in Japan, as documented in Japanese literature cited by Chen *et al*. (2012). A high level of plant diversity has been utilized for more than 100 years, particularly in mountainous villages in Japan. In recent times, land use changes and modernization have led to an important reduction in wild edibles knowledge and availability; consumers' current attitudes towards plant species are still little known (Chen & Qiu 2012).

China is noted for its wide contemporary use of wild components in human diets, probably due to cultural behavior and severe food crises until recently, as mentioned by Kang *et al*. (2012). Research on potentially edible wild plants is well developed and an interesting number of studies are accessible, despite the focus being mainly centered on ethnic minorities (e.g. Mongolians, Shaxi in Sichuan, and Miao in Hunan) rather than in north-central, central, and eastern China, where the dominant Chinese population lives and wild food plant approaches are less well documented (Kang *et al*. 2012).

Using similar methodologies and research efforts, Kang *et al*. (2012, 2013, 2014) found that patterns in wild food plant use in China can be rather different. For instance, they observed that wild vegetables dominate in central China (Kang *et al*. 2012), while fruits formed the largest category in north-west China (Kang *et al*. 2014). Moreover, these authors have registered an impressive number of utilized species of the local edible flora, considering that ethnobotanical studies have been developed at such a small scale. They also reported that people in the Qinling Mountains value forest wild greens over the ruderal taxa, which are still widely used throughout the year and preserved for winter (Kang *et*

al. 2012, 2013, 2014).

Zhang *et al*. (2014) undertook an extensive review of regional literature and found 350 wetland plant species, belonging to 66 botanical families, traditionally used in China, of which 101 species were explicitly used as food and 22 for making liqueurs, altogether corresponding to 35% of the total listed. Ten botanical families contributed nearly 50% (47 species) of all species assigned to food categories; for instance, Polygonaceae, Brassicaceae, and Lamiaceae accounted for 11%, 8%, and 5% of edible species respectively. For liqueur making, Polygonaceae, Poaceae, and Trapaceae represented 54% of the species used (Zhang *et al*. 2014).

Ethnobotanical studies from India (Mir 2014, Panda 2014; Prashanth Kumar & Shiddamallayya 2015) and Pakistan (Abbasi *et al*. 2013a,b,c) also highlight the use of wild plant foods, at times because of their assumed health benefits. Wild fruits and leaves are the best known and consumed plant materials in these regions; some of them are sun dried and stored for several months. Quite a lot of species are described as having more than one edible product, i.e. edible leaves, flowers, fruits, and seeds.

Thirty-nine of the most popular edible plants used in Uzbekistan for improving local diets and helping digestive processes were described by Khojimatova *et al*. (2015). These edible species correspond to 18 families, the most significant being Rosaceae, Amaryllidaceae, and Xanthorrhoeaceae (Chase & Reveal 2009). Analysis of this data shows that some of the reported plants are also used as traditional food in China, Russia, Korea, India, and other countries.

Sometimes, mainly among pastoralist communities, wild foods are consumed as snacks during travels and summer transhumance, as noticed by Boesi (2014). In many cases, nonfood uses of wild edible plants are also relevant; in particular, additional medicinal properties are strongly linked with wild edibles intake (Abbasi *et al*. 2013a,b,c; Uprety *et al*. 2012). This is also the case in Vietnam, studied by Ogle *et al*. (2003), where they have acknowledged the multifunctionality of wild edible plants.

Considering regional biodiversity and availability, in most Asian

regions, the number of inventoried wild greens species is higher than wild fruits, as reported by many researchers (Boesi 2014; Ghorbani *et al*. 2012; Kang *et al*. 2013; Mir 2014, Panda 2014; Prashanth Kumar & Shiddamallayya 2015). However, within other surveys (Kang *et al*. 2014; Li *et al*. 2015 Uprety *et al*. 2012), wild edible fruits are the most cited category (see Table 6.1).

6.2.1.4 Europe

Schulp *et al*. (2014) estimate that 65 million people in Europe (14% of all EU citizens), mainly living in rural areas, collect wild food occasionally (including game, mushrooms, vascular plants), and at least 100 million Europeans consume wild food. Despite these facts, research on wild edible vascular plants does not have the same coverage in all Europe. Countries such as Italy, Spain, and Scandinavia are those where many different studies have been conducted and published (Schulp *et al*. 2014), along with several works developed in Eastern European regions (Łuczaj *et al*. 2013a).

The information summarized by Schulp *et al*. (2014) underlines the use of 592 edible species from 305 genera, identified in 33 studies on wild vascular plant gathering and covering 17 European countries. Most species were reported in one or two countries only, but 81 species are used in four or more countries. Hilly or mountainous areas in central and southern Europe present the highest species richness; lower values are found in agricultural areas, for example in parts of eastern and north-western Europe (Schulp *et al*. 2014).

An interesting overview of changes in the present-day use of wild food plants in Europe, based on examples from different regions, is provided by Łuczaj *et al*. (2012). They confirm a decrease of plant knowledge and contact with nature, but they also discuss that fluctuations in plant use are not linear, because consumption of some species may be linked to temporary needs, habits, and fashions. Besides, they suggest that nowadays in some European countries, wild plants are part of new trends about food, i.e. healthy, good quality, and safe.

Historical ethnobotanical reviews of wild edible plants in Eastern European countries are very good sources of information for comparing earlier and more recent plant use. Records available from Belarus (Łuczaj *et al*. 2013b), Estonia (Kalle & Sõukand 2012), Hungary (Dénes *et al*. 2012), Poland (Łuczaj 2010), Sweden (Svanberg 2012), and Slovakia (Łuczaj 2012) present some ideas about plant resources and patterns of usage in such areas. Moreover, the food use of 175 vascular plant species of the Czech Republic native flora was recently documented by Simkova and Polesny (2015), and Stryamets *et al*. (2015) discussed ethnobotanical and socioeconomic tendencies in wild food collection in rural areas of Russia, Sweden, and Ukraine. Significantly, in most of these studies the use of wild food plants is very similar and characterized by a high interest in wild fruits and seeds and low appreciation of wild greens, which has an important effect on local knowledge and practices, as many available species are not used any more.

In contrast to north-eastern Europe, in the south, coinciding with the Mediterranean area, the consumption of wild vegetables, included leafy greens, is widespread and well represented in traditional and local cuisines (Biscotti & Pieroni 2015; Leonti *et al*. 2006; Tardio *et al*. 2006). Gathering vegetables and fruits in the wild and weeds in disturbed habitats were current practices in southern Europe (Albania, Greece, Cyprus, Malta, Italy, France, Spain, and Portugal), although nowadays they are consumed on a less regular basis (Leonti *et al*. 2006). Despite several ethnobotanical surveys and reviews of food plants covering areas of Italy, Sicily, Spain, Greece, Turkey, and Croatia, the inventory of traditionally gathered wild edibles is still relatively scarce for the Mediterranean basin (Biscotti & Pieroni 2015; Local Food-Nutraceutical Consortium 2005).

The Local Food-Nutraceutical Consortium (2005) project documented 318 wild or semicultivated food plant species (173 species in Spain, 147 in Greece, and 84 in Italy), of which only 18 were used in all the surveyed countries (Leonti *et al*. 2006).

Hadjichambis *et al*. (2008) performed a comparative analysis of the wild food plants recorded by seven selected study sites around the Mediterranean (Albania, Cyprus, Greece, Egypt, Italy,

Morocco, and Spain). They documented 406 wild food plants, corresponding to 294 taxa, of which 77% were used exclusively at a local level, and concluded that even though some species have a general distribution and are commonly used around the Mediterranean, others have a strong connection with local biocultural heritage. Although biological availability is widespread, plant use and traditional knowledge are exclusive to some countries, and the cultural importance of common taxa is very different in each regional gastronomy.

Numerous studies carried out by different researchers contribute to important ethnobotanical, anthropological, socioeconomic, and nutritional information about wild edible plant consumption and associated local knowledge in southern Europe (Dogan *et al*. 2015; Ertug 2000; Ghirardini *et al*. 2007; Guarrera & Savo 2013; Łuczaj & Dolina 2015; Pieroni & Giusti 2009; Pieroni *et al*. 2002; Sansanelli & Tassoni 2014; Turner *et al*. 2011).

Research projects and studies in the Iberian peninsula, particularly in Spain (Alarcón *et al*. 2015; Bonet *et al*. 2002; Carvalho 2010; Carvalho & Morales 2013; González *et al*. 2011; Menendez-Baceta *et al*. 2012; Molina *et al*. 2014; Parada *et al*. 2011; Pardo de Santayana *et al*. 2007; Tardío *et al*. 2006), have reemphasized the cultural and dietary importance of wild edible plants, also strengthening their nutraceutical value, interest as functional foods, and contribution to a healthy diet (Leonti *et al*. 2006; Morales *et al*. 2013, 2014; Sánchez-Mata *et al*. 2012).

Overall, in Europe, Rosaceae, Asteraceae, Brassicaceae, and Ericaceae are the botanical families of wild edible plants most often consumed, among many other locally relevant families such as Apiaceae, Lamiaceae, Amaryllidaceae, and Polygonaceae (Chase & Reveal 2009). Frequently reported categories of plant uses include wild fruits, green vegetables, seasonings, and beverages.

6.2.1.5 Oceania

Literature about the use of wild edible species in Australasia (Australia, New Zealand, and New Guinea) and in the other

archipelagos, islands, and atolls of the Pacific Ocean (Micronesia, Melanesia, and Polynesia) is not easily accessible. Several books focus on the uses of native and introduced plant species that have sustained human life (Balick 2009; Clarke 2011; Cox 1994; Whistler 2001). Searching the main full-text scientific databases may provide some papers on ethnobotanical approaches (Brooker *et al.* 1989; Haberle 2005; Merlin 2000; Sillitoe 1995; Smith 1991), but they are not centered on wild edibles and there are few more recent articles.

Brooker *et al.* (1989) provided an overview on the history of the utilization of New Zealand native flora and mentioned some of the root crops, leafy vegetables, fruits, beverages, seaweeds, and fungi used by the Maori and early settlers. Some examples cited are ferns used as vegetables, like the rootstock of bracken (*Pteridium esculentum* (G. Forst.) Cockayne) and *Blechnum capense* (L.) Schltdl.); the berries from snowberry (*Gaultheria antipoda* G. Forst.), wineberry (*Aristotelia serrata* (J. R. Forst. & G. Forst.) Oliv.), and tree fuchsia (*Fuchsia excorticata* (Forst. & Forst. L. f.); the sea-lettuce (*Ulva lactuca* L.), which is green like ordinary lettuce and was used extensively by the Maori as a vegetable (Brooker *et al.* 1989).

In 1991, Smith combined information from the literature on Aboriginal plant usage in the tropical northern territory of Australia, where people are generally described as having lived on yams, roots, seeds, and fruits, with data from interviews. Fieldwork confirmed that gathering of plant foods was a very important activity in most Aboriginal communities and delivered a list of 148 species used for food. Vegetables, fruits, and seeds were the main food categories mentioned (Smith 1991).

Stewart and Percival (1997) described 30 of the most common bush food plants of New South Wales, Australia. Bush food, also known as bush tucker, is any food native to Australia. Specifically, the bush tucker of plants included fruits, berries, nuts, roots, and greens that sustained Aboriginal existence and promoted a healthy condition, providing a diet rich in vitamins and fibers. Some interesting edible species are the Fabaceae *Acacia aneura* Benth. and *Acacia sophorae* (Labill.) R. Br.; the screwpine, *Pandanus tectorius* Parkinson ex. Du Roi; the Orchidaceae, *Dendrobium*

speciosum Sm.; and the fern *Balantium antarcticum* (Labill.) C. Presl (Stewart & Percival 1997).

The Huli people living in the Tari Basin (above 1500 m altitude) in the Southern Highland Province of Papua New Guinea managed about 67 plant species for food purposes (Haberle 2005).

Foods traditionally eaten within the geographic area known as Remote Oceania were categorized and described by McClatchey (2012), based on the emic classification system of Austronesian languages. The author found three categories of ingredients used in meals: starches (mostly roots and rhizomes), other components (vegetables, meats), and nonmeal foods (raw fruits and raw fish). The majority of species registered are wild foods, and most of these are used as leafy vegetables and fruits. McClatchey suggested in addition that cultural factors such as expectations and preferences may influence the selection and use of plant species, because this author observed that the diversity of wild plants used in Near Oceania (west of Solomon Islands) is greater than in Remote Oceania (Micronesia and Polynesia), even when existing in both areas.

As islands, these areas rely on the sea as an important source of food. There are more than 500 sea plants in the Pacific Islands, and perhaps over 100 of these are locally recognized as being edible (Novaczek 2001). A guide designed to meet the need for community fisheries training, particularly for women, describes some common edible sea plants of the Pacific Islands and compiles useful information about 26 genera, some containing more than one edible species (Novaczek 2001).

6.3 Wild Relatives of Crop Plants

A long transition from foraging to farming began with the harvesting of wild grains and underground organs (roots, tubers, rhizomes, and bulbs). Planting them in permanent mixtures of wild and domesticated types of the same species has been described in many sites of the world. Successful genetic and ecological

approaches provide significant contributions to our understanding of plant evolution and domestication.

According to Harris (2005), "a worldwide distribution of agriculture was mainly the result of expansion from a few core regions where independent transitions from foraging to farming took place at different times, affected by many factors that varied from region to region."

In southern Asia, certain environmental and cultural conditions occurring simultaneously caused some groups of foragers to start cultivating and domesticating a limited range of wild plants. A small selection of seeds from wild legumes and grasses, as well as tubers and roots of some wild plants, were submitted to domestication. These people became the world's first farmers and produced the beginnings of agriculture and horticulture (Harris 2005).

Crop wild relatives (CWR) may be generally defined as wild plant species that are closely related to domesticated plants (Maxted *et al*. 2006). Such species present genetic diversity that has been used to increase crop yields, to obtain new varieties and hybrids, and can also be useful to improve resistance to pests, diseases, and stresses in a changing environment (Heywood *et al*. 2007; Maxted *et al*. 2006). Occasionally, CWR of cultivated plants are not easily determined. Domestication may have been a complex evolutionary process where the assignment of a unique ancestral wild gene pool is problematic (*Milla et al*. 2015). Some crops like leaf mustard (*Brassica juncea* (L.) Czern.) and bread wheat (*Triticum aestivum* L.) have no direct wild progenitors, having occurred via a process of hybridization, even though the origin of the hybrid is not always identified. However, other food species, such as watercress, blackberry (*Rubus* sp.pl.), hazel, carrot, and parsnip (*Pastinaca sativa* L.), are very similar to their wild ancestors, only varying in their edible parts that are particularly well developed (Vaughan & Geissler 2009).

In most regions, several inadvertently or intentionally domesticated wild plant species have become major complementary staples: barley (*Hordeum* L.) and wheat (*Triticum* L.) in south-western Asia; rice (*Oryza* L.) in China;

maize (*Zea mays* L.) in North America; sorghum (*Sorghum bicolor* (L.) Moench) and pearl millet (*Pennisetum glaucum* (L.) R. Br.) in sub-Saharan Africa; herbaceous legumes from the Fabaceae family, represented by lentil, pea, chickpea, and other pulses in south-western Asia, soybean in China, common bean in Mesoamerica, cowpea and groundnuts in West Africa, south of the Sahara. Taro (*Colocasia esculent*a (L.) Schott), yams (*Dioscorea* sp. pl.), bananas (*Musa* sp. pl.), sugarcane (*Saccharum officinarum* L.), and breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) were independently domesticated in New Guinea and south-eastern Asia (Harris 2005).

Zohary (2004), writing about unconscious selection and the evolution of domesticated plants, pointed out that cultivated crops ordinarily maintained by seed propagation (sexual reproduction) and thus passing through consecutive cycles of selection, such as grains and numerous vegetables, diverged considerably from their wild progenitors, being distinguished by complex syndromes of morphological and physiological traits. But, vegetative or clonal propagation (e.g. cutting and grafting), used for perennial fruit trees or corm and tuber crops, taking into account the grower's preferences, fixes desired types of plants/clones that remain relatively close to their wild progenitors. With rare exceptions, selection is completed once a given clone is picked up and most valued genotypes are frequently kept for long periods of time, exhibiting impressive resemblance to the wild forms (Zohary 2004).

In contrast, wild species of direct use for food, in addition to many other purposes (e.g. fodder, medicinal, ornamental, and industrial), did not pass through the genetic limitation of domestication and maintain important genomic features that ensure adaptation to different habitats and biotic and abiotic stresses. Therefore, such wild resources have extended application in plant breeding and are fundamental for improving agricultural and food production, human nourishment, and maintaining sustainable agroecosystems. Nevertheless, some potentially valuable species are threatened in the wild, due to habitat destruction, degradation and fragmentation, conversion of farming systems, overexploitation, invasive flora, and climate change. Survival of many wild plant species that are CWR is at risk from a wide range of drivers of

biodiversity loss, experiencing extensive genetic erosion and even extinction as a result of direct or indirect environmental changes (Heywood 2008, 2011; Heywood *et al*. 2007).

An outstanding contribution to wild and cultivated species germplasm collection and to comprehensive information and use of CWR in plant breeding was achieved by J. R. Harlan (1917–1998) (Hymowitz 1999; Khoury *et al*. 2013). This scientist established the level of domestication of a crop, its perceived genetic vulnerability, as well as the availability of CWR for use, the usability of CWR in research and breeding programs, and the financial, technical, and political circumstances or constraints pertaining to their use (Khoury *et al*. 2013).

Harlan and de Wet (1971) developed a framework for rational classification of cultivated plants. They considered that formal plant taxonomy was not satisfactory for classifying cultivated plants and their wild relatives because taxonomists tended to overclassify and standard botanical categories did not work at infraspecific levels. They studied the total existing set of all genes of a cultivated plant and assigned taxa to one of three gene pools, defining the gene pool concept (Harlan & de Wet 1971). Consequently, close relatives are included in the primary gene pool (GP1), more remote ones in the secondary gene pool (GP2), and very remote ones in the tertiary gene pool (GP3) (Harlan & de Wet 1971).

The gene pool concept has some limitations because in many cases, crossing ability and patterns of genetic diversity between crops and their wild relatives do not exist. Therefore, where crossing and genetic diversity information is lacking, the taxon group concept, using the existing taxonomic hierarchy to recognize the degree of relatedness of a wild species to a crop, may be introduced, although such concept is a more subjective assessment than direct comparison of genetic diversity (Maxted *et al*. 2006).

Nowadays, the most efficient usage of CWR and of wild native or semidomesticated species has an accepted vital role in food security and economic stability and is a matter of global concern, for both more industrialized and the poorest developing regions. A significant number of plant species have been overlooked or

undervalued although they have the potential to provide increased commercial opportunities and improved nutritional status for the population, particularly in Africa, Asia, and Latin America.

Meeting the demands of agriculture, nutrition, and enhancing livelihoods in the twenty-first century involves an appropriate focus on neglected or underutilized species, many of them CWR species, all over the world. International policies and treaties, such as the Convention on Biological Diversity (CBD 2015a), the International Treaty on Plant Genetic Resources (FAO 2009), and the Global Strategy for Plant Conservation (GSPC) (CBD 2015b), recognize CWR conservation as a worldwide priority. The GSPC has a well-defined strategy that includes 16 outcome-oriented global targets set for 2011–2020. Within GSPC Objective II: Plant diversity urgently and effectively conserved, Target 9 specifically proposes "by 2020, 70% of the genetic diversity of crops including their wild relatives and other socioeconomically valuable plant species should be conserved, while respecting, preserving and maintaining associated indigenous and local knowledge" (CBD 2015b). Hence, the essential framework to develop national and regional inventories is already available, as well as networks and information systems to enable the exchange of data related to plant genetic resources for food and agriculture (CBD 2015a,b).

6.3.1 CWR Inventories and Checklists

Crop wild relatives' inventories and checklists of taxonomic diversity and prioritized taxa, at the global, national or regional level, are systematic approaches comprising useful tools for surveying and collecting genetic resources of crop species and wild plants, and also encompassing fundamental strategies for CWR conservation and future use (Maxted *et al*. 2007; Vincent *et al*. 2013).

Maxted *et al*. (2007) describe some of the first global and regional lists of CWR. The preliminary list of European CWR was produced in 1994 by the World Wide Fund for Nature (WWF) and the International Union for Conservation of Nature (IUCN) and extended a year later by Heywood and Zohary who organized a checklist of 206 species and subspecies, focusing on the primary

gene pool of major cultivated species. The following Crop Wild Relative Catalogue for Europe and the Mediterranean (Kell *et al.* 2005) addressed the gene pools of all European socioeconomically important species (Maxted *et al.* 2007), which comprised about 23 483 CWR and 2204 crop taxa (Brehm *et al.* 2008).

At a national level, Maxted *et al.* (2007) cited lists from different European countries provided by several authors: the first CWR inventory for Italy with 163 taxa; a list of 130 CWR taxa for France and another of 44 French wild species representing 23 genera that justified priority conservation; the first comprehensive database of 1603 CWR taxa occurring in Russia; the preliminary list of United Kingdom CWR in 1995, which was expanded in 1999 to include 57 taxa from 26 genera of minor crops that had wild populations present in the UK, but not comprising their wild relatives.

The UK national inventory of CWR contains 413 genera and 1955 species. Approximately 65% of the 2300 UK native taxa are CWR, and of these, 85% are wild relatives of medicinal and aromatic plants, 82% of agricultural and horticultural crops, 15% of forestry plants, and 30% of ornamentals. The botanical families Poaceae, Rosaceae, Fabaceae, Brassicaceae, and Asteraceae present a high level of CWR taxa richness (Maxted *et al.* 2007). A recent publication refers to the English national inventory of priority CWR that contains 148 taxa (126 species and 22 subspecies) (Fielder *et al.* 2015). This number represents 10% of the taxa listed in the checklist of English CWR (reporting 1471 native and introduced taxa) that was developed by matching the previous mentioned UK inventory (Maxted *et al.* 2007), the Catalogue of Crop Wild Relatives for Europe and the Mediterranean, and a list of the English flora, extracted from the Vice County Census (Fielder *et al.* 2015).

Brehm *et al.* (2008) performed a case study on the Portuguese mainland to inventory CWR and wild harvest plants (WHP). They reported 2319 taxa distributed across 524 genera and 122 families. Of the total number, 97.5% are CWR, 21.4% are WHP, 19.0% are both CWR and WHP, and approximately 6.1% are endemic. In Portugal, the top five families of CWR are the Fabaceae, Asteraceae, Poaceae, Lamiaceae, and Caryophyllaceae, accounting

for almost 40% of the total number of CWR taxa. Genera including the highest number of taxa related to food and medicinal use are *Silene* (41 taxa), *Centaurea* (32), *Vicia* (30), *Thymus* (12), *Rumex* (7), *Malva*, *Mentha* and *Polygonum* (6) (Brehm *et al*. 2008).

Wild plant species (CWR and wild utilized species (WUS)) occurring in the United States territory with potential value in crop research and directly used for food and other purposes were compiled from North American databases and floras (Khoury *et al*. 2013). The inventory reported 4596 taxa, representing 3912 species from 985 genera and 194 plant families. CWR (54% of the total taxa) correspond to 1905 species from 160 genera and 56 families; WUS (46%) are represented by 2101 taxa from 2007 species, 833 genera, and 182 families. The botanical families comprising the highest number of species of CWR are Fabaceae (693 species), Poaceae (448), Asteraceae (182), Rosaceae (163), and Amaranthaceae (137) (Khoury *et al*. 2013).

A recent article published by Kell *et al*. (2015) highlights the significant impact of CWR on agriculture, horticulture, and the world economy. Referencing several researchers and using the example of China (one of the most important centers of plant diversity, with more than 30 000 native higher plant species), they emphasize the crucial role of such species in food security and economic stability and report that high-priority native wild relatives are threatened. They also provide a list of 871 high-priority species of the CWR China inventory, within the gene pools of 28 socioeconomically relevant crops to be used for future conservation programs.

Vincent *et al*. (2013) argued that a more systematic and targeted use of CWR is a currently underdeveloped option that could potentially make a significant contribution to increasing food security. The authors described a global priority CWR inventory and list 92 genera of the most socioeconomically important global food crops. Moreover, using preestablished criteria (socioeconomic relevance, potential use, and threatened status) and three main concepts (gene pool, taxon group, and provisional gene pool), they were able to prioritize CWR species covering over 150 crops. They estimated CWR relatedness for priority

crops, documented taxonomy, geographic distribution, potential use, seed storage strategies of valuable CWR, and designed a database available online searchable by crop, gene pool, individual CWR species, country or region (http://www.cwrdiversity.org/checklist/). This checklist is named the Harlan and de Wet CWR Inventory in honor of the scientists who originally proposed the crop gene pool concept (Vincent *et al*. 2013).

The first global list of priority CWR species comprised 1667 taxa, divided between 37 botanical families, 108 genera, 1392 species and 299 subspecific taxa. The families with the most CWR are Fabaceae (253), Rosaceae (194), Poaceae (150), Solanaceae (131), and Rubiaceae (116) while the genera with the most CWR are *Solanum* (124), *Coffea* (116), *Prunus* (102), *Ficu*s (59), and *Ribes* (53). CWR numbers in these lists concern botanical taxa of the major biodiversity and availability of the most important wild edible plants known and consumed by many people worldwide (Vincent *et al*. 2013).

Western Asia with 262 taxa is the region with the highest number of priority CWR, followed by China with 222 taxa and south-eastern Europe with 181. Calculating the unit area per CWR, within the nations with over 80 priority CWR inventoried, the countries with the highest concentration of all priority CWR are Lebanon, Israel, Greece, Portugal, Azerbaijan, Bulgaria, Syria, Italy, Spain, and Turkey. Overall, the countries identified as the highest priority for further CWR targeted conservation initiatives are China, Mexico, and Brazil (Vincent *et al*. 2013).

6.4 Enhancing Biodiversity and Plant Genetic Resources Conservation

Biological diversity or biodiversity is the basis of a sustainable environment and global wellbeing. Biodiversity contributes directly and indirectly to the provision of ecosystem goods and services that correspond to four main categories according to MEA (2005): (i) provisioning services; (ii) regulating services; (iii) supporting services; and (iv) cultural services. Plant use, food strategy and fair, culturally appropriated, ecofriendly, sustainable

diets are intrinsically biodiversity based.

Campbell *et al*. (2012) identified the interlinkages between biodiversity and human wellbeing, i.e. between ecosystems functions and elementary material for good health, security, social relations, and freedom of choice and action. They argued that the recognition of the relations between biodiversity, sustainability, human life and human welfare is a major challenge to contemporary paradigms and support the urgent need for action at national and international levels.

"Plant genetic resources for food and agriculture (PGRFA) consist of diversity of seeds and planting material of traditional varieties and modern cultivars, crop wild relatives and other wild plant species" (AGP 2015). Erosion of these resources contributes to biodiversity loss and poses a severe threat to the world's food security in the long term. Increased environmental awareness of PGRFA erosion has led to a greater demand for conservation measures and transdisciplinary joined-up approaches to assess the implications of global changes and to improve conservation efficiency.

Plant diversity is suffering erosion and extinction at different degrees, which involves both taxonomic and genetic diversity. The level of genetic erosion is not easily estimated as it may go unnoticed because it occurs not only when species become extinct but also in living species. Thus, conservation should focus on local ecosystems protection, as well as on the safeguarding of genetic diversity within the component plant populations (Maxted 2003).

Maintaining PGRFA both in nature (*in situ*) and in gene banks and botanic gardens (*ex situ*) is one of the strategies used to meet conservation goals. It is important to raise public awareness about PGRFA conservation and its contribution to sustainable development of agriculture and the safeguard of biodiversity and agroecosystems.

6.4.1 Conservation Strategies

Conserving plant genetic resources (i.e. PGRFA and wild species) and sustaining biological populations and plants productivity

encompasses technical, ecological, socioeconomic, and cultural factors, and requires successful strategies and appropriate policies.

Technical issues relate to maintaining the full range of genetic variation within a particular species while ecological topics, besides species and populations, are more concerned with natural habitats and agroecosystems, ensuring the ongoing processes of evolution and adaptation within native species' own environments. Plant genetic resources can be conserved both *in situ* and *ex situ*. *In situ* conservation corresponds to the maintenance and recovery of viable populations of species in their natural surroundings. *Ex situ* conservation maintains biological diversity components outside their natural habitats and involves procedures like sampling, transferring, and storing samples of the target taxa (e.g. seeds, propagules, explant cultures, specimens) (AGP 2015). *In situ* management approaches include genetic reserve conservation (e.g. protected areas, such as biosphere reserves, national parks, and wildlife sanctuaries), on-farm conservation (conserving within local farming systems, as farmers have been doing for millennia), and homegarden conservation (crops grown in gardens as small populations and produce used primarily for household consumption). *Ex situ* examples are botanical gardens, gene banks, and field gene banks as living collections. The highest proportion of landraces and CWR diversity is actively conserved *ex situ* (Maxted *et al*. 2011).

The FAO Commission on Genetic Resources for Food and Agriculture (www.fao.org/nr/cgrfa/cgrfa-home/en/) was created in 1983 to deal specifically with issues related to PGRFA. Two important assignments were accomplished during the 1990s: the first report on the State of the World's Plant Genetic Resources for Food and Agriculture, a periodic assessment that delivers a broad overview on the status and trends of conservation and use of plant genetic resources at national, regional, and global levels; and the adoption in 1996 of the Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture, involving 150 countries (AGP 2015). Over the past 20 years, extensive information has become available on genetic erosion and vulnerability of plant genetic resources. Moreover, taking in account the growing demand for new

products, the main drivers of biodiversity loss (e.g. climate change), and major advances in key areas of science and technology (e.g. development of information and communication technologies and of molecular and genomic methods), a second edition of the report on the State of the World's PGRFA (www.fao.org/wiews/en/) was published in 2010. This provided a concise assessment of the status of plant genetic resources and identified the most significant developments, gaps, and needs that were the basis for updating the Global Plan of Action, which was adopted in November 2011 (AGP 2015).

Considering that 2010 was the International Year of Biodiversity and also the year in which the Convention on Biological Diversity failed to meet its major conservation goal of a significant slowdown in biodiversity loss (Heywood 2011), the Second Global Plan of Action, addressing new challenges such as climate change and food insecurity as well as novel opportunities, including information, communication, and molecular methodologies, was fundamental in setting priorities for the effective management of plant genetic resources for the future (AGP 2015). The plan defines 18 priority activities grouped in four main areas: (i) *in situ* conservation and management; (ii) *ex situ* conservation; (iii) sustainable use; and (iv) building sustainable institutional and human capacities (AGP 2015).

Despite significant progresses being made, enhancing biodiversity and plant genetic resources conservation (crops, CWR, and wild species) needs huge commitments to embrace initiatives undertaken under the umbrella of treaties and plans, in order to foster conservation strategies and sustainable use of resources.

At global, national, and regional levels, a number of initiatives have been designed to address conservation issues. Some examples are listed below (AGP 2015).

- *2002: Globally Important Agricultural Heritage Systems (GIAHS)*: such systems are rich in agricultural biodiversity and associated wildlife, linked with local knowledge and experience, reflecting the evolution of humankind and its profound relationship with nature, and are important resources of indigenous knowledge and culture. The GIAHS initiative aims to identify and ensure global recognition of the

importance of these unique traditional agricultural systems for food security and sustainable development, providing dynamic conservation of heritage systems and their multitude of goods and services. GIAHS has project interventions in Algeria, Azerbaijan, Bangladesh, Chile, China, Ethiopia, India, Indonesia, Iran (Islamic Republic), Japan, Kenya, Mexico, Morocco, Peru, Philippines, Sri Lanka, Tanzania, Tunisia, and Turkey (http://www.fao.org/giahs/en/).

- *2004: Global Crop Diversity Trust (GCDT)*: to ensure the conservation and availability of crop diversity for food security worldwide.
- *2005: European Crop Wild Relative Diversity Assessment and Conservation Forum, the PGR Forum Crop Wild Relative Information System (CWRIS)*: the first information management system specifically designed to facilitate CWR conservation and use, developed for Europe and the Mediterranean. It includes taxa (a searchable database of crop species and their associated wild relatives), site and population information, descriptors and links to data on individual species held within other online systems (Heywood 2008; Heywood *et al*. 2007).
- *2006: The Svalbard Global Seed Vault*: an international legal framework for conserving and accessing crop diversity, storing duplicates (back-ups) of seed samples from the world's crop collections. The Vault holds more than 860 000 samples, originating from almost every country in the world.
- *2013: The Millennium Seed Bank of the Royal Botanic Gardens of Kew and the Global Crop Diversity Trust*: a global long-term effort to collect, conserve, and use wild relatives with the characteristics required for adapting the world's most important food crops to climate change. The project Adapting Agriculture to Climate Change is focused on the wild relatives in the gene pools of 29 focal crops (Dempewolf *et al*. 2014).
- *2013: LIBERATION*: linking farmland biodiversity to ecosystem services for effective ecofunctional intensification. Main objectives are to identify general relationships between

seminatural habitats, on-farm management, and biodiversity. Moreover, to link farmland biodiversity to ecosystem services, to value the contribution of ecosystem services for different land-use scenarios, and diffuse information to a wide range of stakeholders.

- *GCP/RAS/240/JPN*: capacity building and regional collaboration for enhancing the conservation and sustainable use of plant genetic resources in Asia.

6.4.2 Promoting and Strengthening Biocultural Heritage

Biocultural heritage is a broad concept overlapping quite a few common interests in understanding the relationship between biological, linguistic, and cultural diversity (Davidson-Hunt *et al.* 2012). It concerns the interactions between people and the natural environment; it is linked with biological resources, from genes to landscapes; it also encompasses long-standing traditions, practices, and knowledge enabling adaptation to different drivers of changes (e.g. environmental, cultural) and challenges (e.g. socioeconomic, demographic). It supports local people's initiatives and dynamic adjustment to meet their own needs and may provide sustainable use of biodiversity.

According to the International Institute for Environment and Development, 370 million indigenous people in the world depend directly on natural resources and still rely on their biocultural heritage for survival (IIED 2015). Since most of the cultural landscapes, wild habitats, agroecosystems, natural resources, crops, and landraces have inherent human management and long-term use, conserving plant genetic resources is highly dependent on the safeguarding of biocultural heritage.

International authorities for nature conservation have been engaged in comprehensive resource networks and operational regulations for protected areas, combining efforts to include local knowledge and skills in contemporary strategies for conserving cultural and ecological diversity. Progress towards greater recognition of indigenous societies and local communities and

their right to reproduce particular knowledge systems and practices differs across the globe. For instance, some European protected areas were legally created to preserve and maintain biological diversity, unique natural features, and associated cultural heritage. However, in some instances the main objectives of such protected areas (e.g. conservation, sustainable development, public use, and community involvement) were not fulfilled, because communication was lacking and participatory approaches were not applied (Carvalho & Frazão-Moreira 2011). Other countries like the United States of America, New Zealand, and Australia have also defined an array of policies and programs to enhance indigenous involvement. Nevertheless, to integrate different priorities and achieve greater inclusion of local people and values is a substantial challenge (Ens *at al*. 2015). In Australia, despite significant contributions to national biological conservation priorities, especially about fire management, threatened fauna and water rights, a general lack of awareness about indigenous history and culture, problems with accepting different knowledge systems, and insufficiently respectful partnerships are the main reasons for limited indigenous involvement in contemporary environmental conservation, with benefits for ecosystem science and management (Ens *at al*. 2015).

To successfully address the loss of both cultural and biological diversity and to achieve effective and fair conservation outcomes, it is fundamental to focus on biocultural approaches to conservation which include new attitudes and integrated programs to balance biodiversity conservation priorities with sustainable human livelihoods.

Gavin *et al*. (2015) argue that the study of biocultural diversity has emphasized the interdependence of biological and cultural diversity via co-evolution processes, common threats, and geographic overlap. They have proposed a set of guidelines and designed a conceptual model for biocultural approaches to conservation assuming that such methodologies are developed within complex social–ecological systems and benefit from previous work on different models of conservation (co-management, integrated conservation and development, and community-based conservation).

It should be stressed that local ecological knowledge and practices are the result of co-evolution over time between humans and their natural environment and are vital to manage resources now and in the future. Plant genetic resources conservation planning and strategies need to respect and combine multiple perspectives and knowledge systems as manifested in many worldviews, languages, and sources of information.

However, one of the most important demands within biocultural approaches to conservation is “to connect local realities with regional and global institutions, bridging gaps and promoting synergies among different sets of knowledge and interests, as well as supporting partnership and prioritizing joint responsibility, active relation management, environmental justice, and the sharing of governance and stewardship responsibility” as accurately suggested by Gavin *et al*. (2015).

6.5 Culturally Significant Wild Edible Plants

Many different botanicals have been used worldwide since ancient times. Within particular geographical and cultural contexts, some species play a role in people’s way of life that sometimes is difficult to estimate. Researchers have attempted to develop methodologies for evaluating the cultural significance of biological taxa in a particular group or culture (Medeiros *et al*. 2011; Pieroni 2001; Reyes-García *et al*. 2006; Tardío & Pardo de Santayana 2008). These approaches measure different dimensions of plants that are relevant to society and provide a more comprehensive evaluation of the significance of floras for humans, avoiding bias and reducing researcher subjectivity (Medeiros *et al*. 2011; Reyes-García *et al*. 2006).

Several surveys within the ethnobotanical literature focus on culturally significant wild plants and associated traditional knowledge, highlighting that local use depends more on the cultural importance of each plant and on the transmission of knowledge and practices needed for using such species than on resource distribution, availability or abundance.

Much of this significance is shaped in local diets, gastronomic traditions, and recipes. Moreover, many edible species also have medicinal properties and spiritual and aesthetic values which strengthen their use. Therefore, as it is an impossible task to mention all culturally significant wild edible species, selected examples from the literature are cited here, trying to give a general overview of some interesting case studies carried out in different geographic regions.

Wild greens with a circum-Mediterranean distribution are highly prized and consumed. Many of the species used belong to the Asteraceae and Brassicaceae families, due to their bitter and pungent taste which is very much appreciated (Biscotti & Pieroni 2015). Golden thistle, *Scolymus hispanicus* L. (Asteraceae), locally known as *cardillo*, is one of the most valued wild vegetables in central Spain (Polo *et al*. 2009). Other thistles also eaten are *Sonchus oleraceus* L. and *Silybum marianum* (L.) Gaertn. (Biscotti & Pieroni 2015; Tardío *et al*. 2006). *Arctium lappa* L., *Cichorium intybus* L., and *Cynara cardunculus* L. are also widely consumed too (Biscotti & Pieroni 2015; Łuczaj 2012; Pieroni *et al*. 2005; Tardío *et al*. 2006). Frequently reported Brassicaceae in Europe are watercress, *Rorippa nasturtium-aquaticum* (L.) Hayek, *Capsella bursa-pastoris* (L.) Medik., wild rucula, *Eruca sativa* L., wild mustard, *Sinapsis arvensis* L., and wall-rocket, *Diplotaxis tenuifolia* (L.) DC. (Biscotti & Pieroni 2015; Tardío *et al*. 2006).

Herbal teas or tisanes are very popular in many countries across central Europe as observed in a survey conducted in 29 different areas (Sõukand *et al*. 2013). Tisanes are drunk in a food context, apparently without any medicinal purpose. Results highlight that representative botanical families used to prepare herbal teas are Lamiaceae and Asteraceae in all studied areas, and Rosaceae only in eastern and central Europe. The main taxa are *Matricaria*, *Mentha*, *Origanum*, *Tilia*, *Thymus*, and *Rubus*. At a regional level, *Rubus idaeus* L. is the most used in eastern Europe, *Chamaemelum nobil*e (L.) All. in southern Europe and *Rosa canina* L. in central Europe (Sõukand *et al*. 2013).

Amaryllidaceae and Asparagaceae are mostly perennial bulbous or

rhizomatous herbaceous plants. Several species from these families are of great importance as wild food in the Mediterranean and Asia; for instance, wild specimens from the genus *Allium* (Kang *et al*. 2013; Pieroni *et al*. 2005; Tardío *et al*. 2006), *Leopoldia comosa* (L.) Parl. (Biscotti & Pieroni 2015; Pieroni *et al*. 2002) and *Asparagus acutifolius* L. (Biscotti & Pieroni 2015; Tardío *et al*. 2006).

According to most recent taxonomical approaches supported by both morphological and phylogenetic analyses, the Amaranthaceae is a broadly defined botanical family that includes plants formerly treated as Chenopodiaceae (APG III 2009; Chase & Reveal 2009). The new Amaranthaceae family comprises approximately 180 genera and 2500 species, mainly from tropical Africa and North America (APG III 2009). Genera including *Amaranthus*, *Gomphrena*, *Beta*, *Chenopodium*, *Atriplex*, *Salsonia*, and *Spinaca* are spread throughout the world in wild and domesticated forms. Wild amaranth seeds (genus *Amaranthus*) were gathered by many Native American people for food and ritual purposes. Leaves and seeds are sources of high-quality protein and the plants grow like a weed in many different environments in the Americas, Africa, and Asia (Vaughan & Geissler 2009).

Six endemic species of wild yam (*Dioscorea* sp. pl.) were identified as potential food resource in the Mahafaly region, south-western Madagascar. Wild yam tubers are used as a staple food by 42% of households close to forest areas, where daily plant collection is accessible. Cassava, maize or sweet potato may be substituted. Different types are identified by local people who prize their sweet taste, size of tubers, and claimed nutritional value. Wild yams have a central role in local food security in the Mahafaly region, especially for poor farmers (Andriamparany *et al*. 2014).

Based on a literature survey, in South Africa Bvenura and Afolayan (2015) found several plant species with great potential to reduce food insecurity at a regional scale. Despite some toxicity problems, the fruits are edible and tender shoots and leaves may be eaten raw or cooked or dried for later use. These species were Spanish needle, *Bidens pilosa* L. (Asteraceae); bastard mustard,

Cleome gynandra L. and *C. monophyla* L. (Brassicaceae); Jew's mallow, *Corchorus tridens* L. and *Corchorus olitorius* L. (Malvaceae); balsamina, *Momordica balsamina* L. (Cucurbitaceae); and black nightshade, *Solanum nigrum* L. (Solanaceae).

Several authors have described particular usages of some edible wild plants that highlight specific issues in addition to dietary or nutritional interest.

- Ertug (2000) gave information about vegetables for preparing *yufka* (greens eaten raw with salt and bread) and *cacik* (vegetables chopped and cooked with onions and bulgur, usually eaten with yogurt) in Anatolia, Turkey.
- Pieroni *et al*. (2002) analyze the use of *liakra* (leaves of weedy greens) by Albanian descendants in southern Italy and discuss a rich heritage under the multidisciplinary perspectives of ethnobotany, ethnotaxonomy, ethnoecology, and ethnopharmacology.
- Nabel *et al*. (2006) document the uses of *ta chòrta* (wild edible greens) in southern Calabria, Italy, where local inhabitants regularly gather more than 40 wild food species.
- Dogan *et al*. (2012) identify 87 botanical taxa, mainly wild and belonging to 27 families, used to prepare *sarma* (cooked leaves for wrapping rice or meat) in Turkey and the Balkans.
- Cruz *et al*. (2014), through 12 species in a rural area of the Caatinga, Brazil, evaluated people's perceptions regarding the use of wild edible plants and found that cultural acceptance, flavor, and emergency food were significantly associated with consumption.
- Kang *et al*. (2014) record the use of *cai* by the Tibetans of Gongba Valley, China. Wild vegetables are usually boiled and/or fried and served as side-dishes (*cai*) but they are also dried for further use or lacto-fermented in wooden barrels.
- Hong *et al*. (2015) describe processing procedures of *jiuqianjiu* liquor, made from water, rice, and a special starter of wild plants known as *xiaoqu* in Sandu Shui County of

Guizhou, China. They report 103 wild-harvested plant species used as starters for preparing fermented alcoholic beverages.

- Sõukand *et al.* (2015) report botanical diversity (116 taxa from 37 families) used to make fermented foods and beverages in seven eastern European countries, upon which further microbiological, nutritional, and pharmacological studies may be developed to address their rational use. Moreover, the authors also list the most uncommon and endangered preparations.

6.6 Conclusion

The consumption of wild edible plant species is not easy to estimate. There have been some attempts to assess the real macro- and micronutrient intake of such components of several food systems, but detailed systematic transdisciplinary studies on edible wild plants are still required, contributing to overcome the world's nutrition problems and to understand the remaining unknown roles of wild edible plants in food security, local diets, and within many groups and societies worldwide.

Wild plant foods have been important sources of nutrients in the past. However, even now, many people rely on these foods to satisfy basic nutritional needs, particularly in underdeveloped regions where undernourishment prevails, due to wide socioeconomic differences persisting in many areas of the world.

Many countries have failed to reach the international hunger targets. Natural disasters and sociopolitical instability have resulted in prolonged crises with increased vulnerability and food insecurity for large parts of the world population (FAO 2015).

Research on wild edibles use goes beyond dietary approaches. Wild foods and local gastronomies are representations of traditional ecological knowledge locally managed and transmitted over centuries by many generations. This knowledge encompasses skills in managing habitats and using resources in a sustainable way.

In indigenous territories, as well as in isolated mountain areas or

rural agricultural landscapes, wild edibles are a symbol of precise identity and cultural heritage. Wild edible plants are versatile and thus are used within cultural environments, as foods and medicine in addition to many other purposes, such as building, fibers, wood, fodder, dye, rituals, and religious festivals.

Technical entities and governance have undervalued wild edible plants; they have been considered minor species or weeds to be eradicated from cropland. This perspective, along with global societal changes, has led to loss of the ability to identify and consume the available diversity of wild plant resources. Moreover, deforestation and overexploitation, conflicts, climate changes, and natural disasters have also threatened natural resources worldwide.

Different conservation strategies are required to address erosion of both cultural and biological diversity. Sustainability in wild plant gathering is also a relevant topic to overcome in some specific cases (e.g. underground organs and massive harvesting).

Biocultural approaches to conservation can achieve effective outcomes and successfully deal with cross-cultural awareness and communication challenges, bridging local communities and biologists, environmental managers and policy makers.

Food systems embody resources, ingredients, culture, values, and identity. This chapter does not intend to be an exhaustive approach, but to enhance the perception of the many dimensions of edible wild plants, while emphasizing the conservation of biocultural heritage and stressing the importance of undertaking further transdisciplinary research.

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7
Wild Greens as Source of Nutritive and Bioactive Compounds Over the World

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7.1 Introduction

The use of edible greens as food is as old as civilization. In the past, most people living in rural communities knew about a wide variety of wild plants that they used for many purposes, often linked to survival (food or medicinal uses; see Chapter 6). Many wild plants have been eaten and knowledge of how to identify them, the optimal moment of consumption, methods of preparation, and their uses was acquired by trial and error, and passed down through the centuries.

Agricultural activities have lead to a loss of diversity of plants consumed, and in the last century drastic changes in life styles (migrations, changes in food habit, etc.) have also lead to the loss of a vast part of this age-old knowledge. Since the 20th century, some efforts have been made to recover it: scientists started to study the potential of wild plants as sources of nutrients as well as active principles for medicinal use; renewed interest in a lifestyle more integrated with nature has arisen in some parts of society; ethnobotanists and ethnozoologists have worked on compilation of traditional knowledge about the use of natural resources. In this context, some governments and international organizations (such as UNESCO or the World Intellectual Property Organization), perceiving the importance of preserving this richness, have funded some of these initiatives, and many strategies have been followed in order to revalorize the nutritional potential of wild edibles for

improving the quality of modern diets. Knowledge of the nutrients and bioactive compounds in these traditionally used plants is a key point, which is currently gaining importance in the field of food chemistry.

7.2 Wild Greens as a Source of Nutritive and Bioactive Compounds in Different Geographical Areas

7.2.1 Traditional Wild Greens from Africa

The African continent has a special profile when compared to other world areas in terms of the state of knowledge about wild edible greens composition. The geographical situation of this continent with respect to the equator is reflected in its climatic zonation: from the Mediterranean regions of the North, through subtropical, tropical, and again subtropical areas in South Africa. This area includes many different types of ecosystems such as forests, savannahs, jungles, and deserts, covering more than 30 million km^2, with a wide biodiversity of wildlife (Griffiths 2005).

The degree of socioeconomic development and the historical and cultural circumstances of African countries (for example, the influence of European colonization) have influenced local food habits. Great diversity is found, from extreme poverty in sub-Saharan Africa to the high degree of development of South Africa, passing through different grades of development in other areas. The countries in the north, bordering the Mediterranean, have a greater influence from Europe while those to the east may be more influenced by Asia (Chabal 2001).

Geographical location and distance to fresh produce markets, season of the year, age and gender, ethnicity or religion may all influence food habits. For example, religious celebrations or rituals may be accompanied by eating specific meals with indigenous ingredients and autochthonous vegetables. Considering all these

circumstances, Jansen van Rensberg *et al*. (2007) showed that in South Africa, poor households use wild leafy vegetables more than wealthier ones. In another study undertaken in Uganda by Tabuti *et al*. (2004), the consumption of wild plants was limited to casual encounters, periods of food shortages, and as supplements to major food crops.

In this context, a wide variety of plants is used in daily life for food, water (for example, watermelons have been used as a source of water in dessert), shelter, firewood, medicine, and other necessities (van Wyk & Gericke 2000), from the ancestral traditions of indigenous people to the present day. The River Nile region and eastern Africa are among the earliest places where humans experimented with primitive food production strategies including hunting, gathering, and primal cultivation (Brandt 1984; Hadidi 1985).

Thus, the African population has a long history of using indigenous leafy vegetables, which contribute significantly to household food security and add variety to cereal-based staple diets. These vegetables are often generically called "spinach," are gathered predominantly by women, and may be eaten raw, cooked, or together with starchy foods (for example, in a porridge). A single plant species may be eaten or a combination of different species, alone or mixed with other ingredients, such as oil, butter, groundnuts, coconut, milk, tomato or onion (Uusiku *et al*. 2010). For many, the traditional names are indicative of the fact that they are usually eaten; for example, *Lanatana trifolia* L., traditionally eaten in Ethiopia, is called "yerejna kollo" in the Amharic language, which means "shepherd's snack" (Asfaw & Tadesse 2001).

Despite these ancient practices, many African autochthonous vegetables have become underutilized in favor of introduced nonnative vegetables (such as spinach or cabbage, among others); however, some studies show that indigenous species, when available, are still preferred to other exotic vegetables (Marshall 2001). The decline in the use of indigenous vegetables by many rural communities has resulted in poor diets and increased incidence of nutritional deficiency disorders in many parts of Africa (Odhav *et al*. 2007).

The main nutritional problem in many of these areas is chronic undernutrition, affecting some 200 million people. Sub-Saharan Africa has the highest prevalence of undernutrition in the world: one-third of the population is chronically hungry, the majority of whom live in rural areas, and high numbers of children are suffering the consequences of this problem. Food problems affect each country differently, the dryer Sahelian countries being more prone to food shortages and starvation than forested ones (Lopriore & Muehlhoff 2003). Malnutrition in Africa manifests as protein-energy malnutrition, but also as vitamin and mineral deficiencies.

Africa has the highest prevalence of anemia and vitamin A deficiency in the world; these are two of the three major micronutrient deficiencies recognized by the WHO (iron, iodine, and vitamin A), and considered as a moderate-to-severe public health problem in most African countries, especially those in the central part of the continent, where almost half the population are affected by one of these deficiencies. According to WHO data (Benoist *et al*. 2008), 67.6% of preschool-aged children (<5 years old), 57.1% of pregnant women, and 47.5% of nonpregnant women of fertile age are affected by anemia (meaning more than 83, 17, and 69 million individuals affected, respectively). The prevalence of vitamin A deficiency in Africa is around 42%, with more than 56 million preschool-aged children and more than 4 million pregnant women suffering biochemical vitamin A deficiency (low levels of serum retinol), as well as 2.5 million preschool-aged children and 3 million pregnant women suffering from night blindness (WHO 2009). It is estimated that over 228 000 deaths of children under five which occur each year in the Economic Community of West African States (ECOWAS) countries (Benin, Burkina Faso, Cape Verde, Côte d'Ivoire, The Gambia, Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Mauritania, Niger, Nigeria, Senegal, Sierra Leone, and Togo) are attributable to vitamin A deficiency (Aguayo 2005; Sifri *et al*. 2003).

Regarding other nutrients, with the exception of relatively small local surveys, there are insufficient data to make a reliable estimation of the prevalence of their deficiency in the world, as their adverse effects on health are sometimes nonspecific and the

public health implications are less well understood. However, national survey data from a few countries suggest that deficiencies of zinc, calcium, folate or vitamin D make a substantial contribution to the global burden of disease (Allen *et al*. 2006).

Also fiber intake is limited, which is aggravated by the migration of communities from rural areas to cities, often introducing a diet with high sugar and fat and low fiber content. A study undertaken by Ruel *et al*. (2005) on 10 sub-Saharan countries showed that none of them reached the WHO/FAO recommended minimum daily intake of fruits and vegetables. A diversified diet would be needed to meet the daily micronutrient requirements, and particularly, diets low in fiber and micronutrients could be improved with a higher intake of fruits and vegetables; these foods have also been shown to provide many bioactive compounds of great interest in the prevention of diet-related diseases. In this context, traditional vegetables grow wild and readily available in the field; as they are autochthonous species adapted to soil and climate, they do not need formal cultivation techniques, such as water supplies (an important problem in many areas) or other agricultural strategies often needed for horticultural crops.

Although the food use of wild greens by indigenous African populations is well known, their socioeconomic situation has delayed the gathering of scientific knowledge about this topic. The analysis of the chemical composition of African wild edible or medicinal species, in terms of nutrients, bioactive compounds or pharmacological activities, is recent. Many analyses focus on the medicinal properties of these wild plants or their essential oils, testing traditionally known properties or searching for medicinal applications.

The first studies about nutritional value of indigenous African wild vegetables were published in the late 1970s, for example that of Saleh *et al*. (1977) in Egypt. In the last 20 years some work has been conducted, mainly in South Africa (Afolayan & Jimoh, 2009; Kruger *et al*. 1998; Nesamvuni *et al*. 2001; Odhav *et al*. 2007; Steyn *et al*. 2001). Studies undertaken in the Mediterranean countries of North Africa often focus mainly on the study of wild plant essential oils, aromatic plants and spices, as well as wild fruits composition (for example, Boudraa *et al*. (2010) in Argelia,

Akrout *et al*. (2012) in Tunisia, Imelouane *et al*. (2011) and Rsaissi *et al*. (2013) in Morocco) rather than wild greens (Tlili *et al*. (2009), conducted in Tunisia). Only a few works have been published on the nutritional composition of wild leafy vegetables, from Nigeria (Isong & Idiong 1997; Lockett *et al*. 2000), Ghana (Wallace *et al*. 1998), Malawi (Mosha & Gaga 1999), Senegal (Ndong *et al*. 2008), Cameroon (Bouba *et al*. 2012) and Kenya (Orech *et al*. 2007).

Wild greens usually have an energy value and proximal composition close to cultivated vegetables, with 3–10 % of available carbohydrate content. Odhav *et al*. (2007) reported values near 10% for *Physalis viscosa* L., *Senna occidentalis* (L.) Link or *Solanum nodiflorum* Jacq. aerial parts, gathered in South Africa. Some exceptions of leafy vegetables especially rich in carbohydrates can be found, such as the leaves of *Manihot esculenta* Crantz., with 18 g/100 g, or *Adansonia digitata* L. (baobab), with 16 g/100 g (FAO 1990; Kruger *et al*. 1998). Baobab leaves are rich in mucilage and widely used in soups as a vegetable in tropical Africa, or sun-dried, ground and powdered ("lalo") for seasoning in West Africa (FAO 1988). Lipid content is usually below 1%, with some exceptions such as *Centella asiatica* L. Urb., *Ceratotheca triloba* (Bernh.) E. May. ex Bernh., and *Senna occidentalis* (around 2% according to Odhav *et al*. (2007)). In some cases, wild leafy vegetables may have a considerable protein content, up to 2–7%, higher than the protein content of many commercial vegetables with the exception of certain legumes, as reported by Odhav *et al*. (2007) and Kruger *et al*. (1998). The leaves and shoots of some *Moringa* species (horse radish, eaten raw in salads like cress or cooked as a vegetable) are a good example, showing around 6–7 g of protein per 100 g of fresh plant, with high levels of the essential amino acid methionine (FAO 1988; Yang *et al*. 2006). Although plant proteins are not high quality in terms of covering essential amino acid needs, they could still provide a contribution to the highly deficient protein intake in many African populations. Carbohydrates, lipids, and proteins are the main contributors to energy value, ranging around 50–300 kcal/100 g fresh weight (fw) (Odhav *et al*. 2007; Uusiku *et al*. 2010), which is not a high value taking into account the high

energy requirements of undernourished populations.

However, the main contribution of wild greens to the African diet is in terms of micronutrients and bioactive compounds. Table 7.1 presents data on the relevant levels of vitamins and minerals in wild leafy vegetables traditionally consumed in Africa obtained from scientific literature. Only data on species with significant nutrient content have been recorded: 100 g of fresh material providing more than 15% of generally accepted daily recommendations such as the FNB (Food and Nutritition Board) of the American Institute of Medicine (Trumbo *et al*. 2002) or FAO/WHO (2004). However, many other species have been traditionally eaten and reported as good contributors to the human diet; a wide variety of them are compiled in FAO (1988).

Table 7.1 Leafy vegetables traditionally consumed in Africa, standing out as sources of vitamins or minerals. Data are given per 100 g of fresh weight.

Species	Vitamins	Minerals	References
Adansonia digitata L.	Vitamin C (52 mg/100 g)	Ca (208–518 mg/100 g); Fe (9.59 mg/100 g); Mn (0.56 mg/100 g)	FAO 1990; Lockett *et al*. 2000
Amaranthus spp. (*A. viridis* L., *A. caudatus* L., *A. gracilis* Desf., *A. hybridus* L., *A. dubius* Mart., *A. spinosus* L.)	RE* (327 µg/100 g); vitamin B_9 (64 µg/100 g); vitamin C (22–126 mg/100 g)	Ca (253–425 mg/100 g); Mg (105–268 mg/100 g); Fe (0.5–9.8 mg/100 g); Zn (1.35–8.4 mg/100 g); Mn (0.27–12.3 mg/100 g)	FAO 1990; Kruger *et al*. 1998, Nesamvuni *et al*. 2001; Steyn *et al*. 2001; Odhav *et al*. 2007
Asystasia gangetica L.	–	Ca (385 mg/100 g); Mg (144 mg/100 g); P (122	Odhav *et al*. 2007

		mg/100 g); Fe (2.55 mg/100 g); Mn (2.7 mg/100 g)	
Bidens pilosa L.	RE* (301–985 µg/100 g); vitamin B_9 (351 µg/100 g); vitamin C (23 mg/100 g)	Ca (162–340 mg/100 g); Mg (79–157 mg/100 g); Fe (2–6 mg/100 g); Cu (1.2 mg/100 g)	FAO 1990; Kruger *et al.* 1998; Odhav *et al.* 2007
Brassica spp.	Vitamin C (30–113 mg/100 g)	–	Kruger *et al.* 1998; Mosha & Gaga 1999
Ceratotheca triloba (Bernh.) E.May. ex Bernh.	–	Fe (2.9 mg/100 g); Mn (1.2 mg/100 g)	Odhav *et al.* 2007
Centella asiatica Urb.	–	Ca (291 mg/100 g); Mg (32.5 mg/100 g); Fe (2.16 mg/100 g); Mn (2.76 mg/100 g)	Odhav *et al.* 2007
Chenopodium album L.	RE* (917 µg/100 g); vitamin C (31 mg/100 g)	Ca (250–330 mg/100 g); Mg (109–211 mg/100 g); Fe (2.1–6.1 mg/100 g); Zn (1.4–18.5 mg/100 g); Mn (1.8–4.6 mg/100 g)	Kruger *et al.* 1998; Odhav *et al.* 2007; Afolayan & Jimoh 2009
Cleome spp.	RE* (663–	Ca (206–384	FAO 1990;

(*C. gynandra* L., *C. monophylla* L.)	1536 μg/100 g); vitamin B_9 (346–418 μg/100 g); vitamin C (13–50 mg/100 g)	mg/100 g); Mg (44–76 mg/100 g); Fe (2.6–9.7 mg/100 g); Mn (1.2 mg/100 g)	Kruger *et al.* 1998; Nesamvuni *et al.* 2001; Odhav *et al.* 2007
Corchorus olitorius L.	vitamin C (38 mg/100 g); RE (966 μg/100 g)	Fe (2 mg/100 g)	Saleh *et al.* 1977; Orech *et al.* 2007
Corchorus tridens L.	RE* (533 μg/100 g)	Ca (363 mg/100 g); Mg (73 mg/100 g); Fe (11.5 mg/100 g)	Nesamvuni *et al.* 2001
Cucumis metuliferus Naudin	–	Ca (386 mg/100 g); Mg (132 mg/100 g); Fe (2.4 mg/100 g); Mn (0.52 mg/100 g)	Odhav *et al.* 2007
Cucurbita pepo L.	RE* (194 μg/100 g); vitamin C (11 mg/100 g)	–	Kruger *et al.* 1998
Emex australis Steinh.	–	Mg (1.7 mg/100 g); Zn (2.2 mg/100 g); Mn (3.41 mg/100 g)	Kruger *et al.* 1998; Odhav *et al.* 2007
Euphorbia hirta L.	–	Ca (176 mg/100 g); Zn (5.29 mg/100 g)	Wallace *et al.* 1998
Ficus thonningii Blume	–	Ca (285 mg/100 g); Mg (45.3 mg/100	Lockett *et al.* 2000

		g); Fe (5.12 mg/100 g); Mn (1.33 mg/100 g)	
Galinsoga parviflora Cav.	–	Fe (3 mg/100 g)	–
Grewia occidentalis L.	–	Ca (183 mg/100 g); Mg (130 mg/100 g); P (76 mg/100 g)	Steyn *et al.* 2001
Hibiscus trionum L.	–	Ca (2171 mg/100 g); Mg (731 mg/100 g); P (290 mg/100 g); Zn (5.7 mg/100 g)	Steyn *et al.* 2001
Hibiscus manihot L.	–	Ca (322 mg/100 g); Mg (244 mg/100 g); Fe (141 mg/100 g)	Aalbersberg *et al.* 1991
Ipomoea batatas (L.) Lam.	RE* (103–980 µg/100 g); vitamin B_9 (80 µg/100 g); vitamin C (11–70 mg/100 g)	Mg (61 mg/100 g)	FAO 1990; Kruger *et al.* 1998; Mosha & Gaga 1999
Justicia flava Vahl.	–	Ca (331 mg/100 g); Mg (225 mg/100 g); Fe (2.6 mg/100 g); Zn (1.8 mg/100 g); Mn (1.3 mg/100 g)	Odhav *et al.* 2007
Manihot	RE* (1970	–	FAO 1990;

esculenta Crantz	µg/100 g); vitamin B_9 (80 µg/100 g); vitamin C (311 mg/100 g)		Kruger *et al*. 1998
Momordica balsamina L.	–	Ca (403 mg/100 g); Fe (3.4–3.5 mg/100 g); Zn (1.8 mg/100 g); Mn (1.5 mg/100 g)	Odhav *et al*. 2007
Moringa oleifera Lam.	–	Ca (111–141 mg/100 g); Fe (4.3–5.7 mg/100 g); Mn (0.46 mg/100 g)	Lockett *et al*. 2000; Ndong *et al*. 2008
Moringa peregrina Fiori	RE* (400 µg/100 g); vitamin C (264 mg/100 g)	Ca (458 mg/100 g); Fe (5.6 mg/100 g)	Yang *et al*. 2006
Moringa foetida Schumach	RE* (901 µg/100 g); vitamin C (20 mg/100 g)	–	Nesamvuni *et al*. 2001
Opuntia microdasys (Lehm.) Pfeiff	–	Ca (2.4 mg/100 g); Mg (5.8 mg/100 g); K (10 mg/100 g)	Chahdoura *et al*. 2015
Opuntia macrorhiza (Engelm.)	–	Ca (3 mg/100 g); Mg (1.6 mg/100 g); K (4.3 mg/100 g)	Chahdoura *et al*. 2015
Oxygonum sinuatum	–	Mg (41.6 mg/100 g); Fe	Odhav *et al*. 2007

Dammer		(3.12 mg/100 g)	
Physalis viscosa	–	Mg (101 mg/100 g); Fe (3.8 mg/100 g)	Odhav *et al.* 2007
Portulaca oleracea L.	–	Fe (2.9 mg/100 g); Zn (2.4 mg/100 g); Mn (1.68 mg/100 g)	Odhav *et al.* 2007
Rumex spp.	Vitamin C (121 mg/100 g)	–	Saleh *et al.* 1977
Scandicium stellatum Thell.	Vitamin C (196 mg/100 g)	–	Saleh *et al.* 1977
Senna occidentalis (L.) Link.	–	Ca (513 mg/100 g); Mg (1.61 mg/100 g); Fe (2.5 mg/100 g); Zn (2.1 mg/100 g)	Odhav *et al.* 2007; Kruger *et al.* 1998
Sonchus asper (L.) Hill.	–	Ca (344 mg/100 g); Mg (69.8 mg/100 g); Fe (22.4 mg/100 g); Mn (1.17 mg/100 g)	Afolayan & Jimoh 2009
Sonchus oleraceus L.	Vitamin C (67 mg/100 g)	Ca (193 mg/100 g); Mg (50.7 mg/100 g); Fe (14.9 mg/100 g)	Saleh *et al.* 1977; Steyn *et al.* 2001
Spinacia oleracea L.	RE* (669 µg/100 g); vitamin B_9 (194 µg/100 g);	Mg (79 mg/100 g); Fe (2.7 mg/100 g)	Kruger *et al.* 1998; Steyn *et al.* 2001

	vitamin C (28 mg/100 g)		
Trigonella foenum-graecum L.	Vitamin C (207 mg/100 g)	–	Saleh *et al.* 1977
Urtica urens L.	–	Mg (292 mg/100 g); Mn (4.45 mg/100 g)	Kruger *et al.* 1998; Odhav *et al.* 2007
Vernonia spp.	Vitamin C (51–198 mg/100 g)	Ca (123 mg/100 g); Fe (3.5 mg/100 g); Mn (0.69 mg/100 g)	FAO 1990; Lockett *et al.* 2000; Ejo *et al.* 2007
Vigna unguiculata (L.) Walp.	RE* (99 µg/100 g); vitamin B_9 (141 µg/100 g); vitamin C (50 mg/100 g)	Ca (188 mg/100 g); Mg (60 mg/100 g)	Kruger *et al.* 1998
Wahlenbergia undulata A. DC.	–	Ca (38.6 mg/100 g); Fe (3.8 mg/100 g); Mn (1.4 mg/100 g)	Kruger *et al.* 199;, Odhav *et al.* 2007

* RE (= retinol equivalents) are usually calculated as (content of β-carotene/6) + (contents of other provitamin A carotenoids /12), according to Mahan *et al.* (2012).

From a nutritional point of view, minerals can be divided into macroelements and microelements. Among macroelements, calcium is one of the most important, since a deficiency in calcium intake in infants may induce rickets, while in the elderly it leads to the development of osteoporosis and tetany in skeletal muscles (Mahan *et al.* 2012; Schrager 2005). High calcium intake should be achieved during the development of bone mass, in the earlier stages of life. A dietary calcium range of 210–800 mg/day is recommended for infants and younger children, while adults need

700–1000 mg/day (Cuervo *et al*. 2009). These levels take into account factors affecting calcium bioavailability, such as individual conditions, as well as the form present in the food, and the presence of components enhancing or decreasing its absorption.

Although leafy vegetables contain abundant calcium, with levels even higher than many foods widely accepted as good calcium sources, such as dairy products, they are also one of the main sources of oxalates in the diet, which are assumed to have a negative impact on mineral absorption due to their ability to bind free minerals in the small intestine, forming insoluble oxalates that remain nonabsorbed in the gut. Generally, oxalic acid may reduce calcium absorption by about one-sixth, so foods with a ratio of oxalic acid/Ca lower than 2.5 are preferably for humans (Concon 1988; Derache 1990; Mahan *et al*. 2012).

To the authors' knowledge, there is little published information about oxalate content in African wild leafy vegetables; however, some species growing in Africa are known to contain high oxalate levels (for example, *Portulaca oleracea* L., *Chenopocium album* L., some *Rumex* spp. or *Amaranthus* spp. leaves) (Bianco *et al*. 1998; FAO 1988; Morales *et al*. 2014; Sánchez-Mata & Tardío 2016). Ilelaboye *et al*. (2013) reported levels below 50 mg/100 g of oxalates, 84–313 mg/100 g of phytates, and less than 10 mg/100 g of tannins in leaves of African *Amaranthus hybridus* L., *Colocasia esculenta* Schott., *Solanum nigrum* L., *Telfairia occidentalis* Hook. f., or *Crassocephalum crepidioides* S. Moore, with good oxalic acid/Ca ratio. When cooking these species, minerals were heat stable, but they lixiviated to the cooking liquid, as well as antinutrients such as oxalates, phytates or tannins, as shown by Ilelaboye *et al*. (2013).

Even taking into account the presence of these antinutrients with their ability of complexing mineral elements, many wild African species stand out for their very high calcium levels, which, although not totally bioavailable, may be considered as an interesting contribution in a diet that is poor in dairy products for different reasons (low access to milk and high prevalence of lactose intolerance in the African population) (Lomer *et al*. 2008; Pettifor 2004).

Vegetables such as *Adansonia digitata* L., *Amaranthus* spp., *Momordica* spp. or *Senna occidentalis* (L.) Link stand out (see Table 7.1), with calcium levels higher than 400 mg/100 g of fresh product, meaning that, with a 100 g portion of these greens, nearly half of the daily recommended levels of calcium for adults could be achieved, and this ratio would be even higher for infants. There are not many data about oxalate levels in the leaves of these species but in some cases, they have exhibited low levels (as previously mentioned for *Amaranthus hybridus*). According to the FAO (1988), calcium present in fresh young baobab leaves (*A. digitata*) may be better absorbed than that from other greens. *Hibiscus trionum* L. is also remarkable, reaching 2 g of calcium/100 g (see Table 7.1).

Regarding other macroelements, Table 7.1 shows that magnesium is abundant in *Amaranthus* spp., *Asystasia gangetica* T. Anderson, *Bidens pilosa* L., *Chenopodium album*, *Grewia occidentalis* L. and *Justicia flava* Vahl edible parts.

Iron is also a very important element for the diet of African populations, since high iron losses or low iron intakes cause anemia, with infants below two years old, adolescent girls, and pregnant women being the main groups at risk. Most institutions recommend 8–10 mg Fe/day for men and elderly women, and about 16–20 mg/day for women below 50–55 years old due to menstruation (Cuervo *et al*. 2009).

Iron is present in foods either as heme Fe in animal tissues or nonheme Fe (inorganic) in plant tissues, such as legumes and vegetables. The former is more easily absorbed (bioavailability of 20–30%), while only 2–10% of inorganic Fe is absorbed (Bothwell *et al*. 1989). Despite the poor absorption of plant origin iron, it represents a contribution that should be taken into account in populations with difficult access to animal origin foods such as meat. Iron sources with easy accessibility such as autochthonous plants are thus of great importance in improving the quality of the African diet.

In this respect, *Adansonia digitata* and *Amaranthus* spp. edible parts may reach almost 10 mg Fe/100 g fw, which is a similar level to those found in legume seeds or vegetables traditionally

considered as good iron sources such as spinach (around 2–4 mg/100 g, according to Souci *et al*. (2008)). Also, some *Sonchus* species have been shown to present 14–23 mg Fe/100 g, a very high level for a plant food. Van der Walt *et al*. (2009) indicate a very high value of iron in samples of *Amaranthus thunbergii* Moq. leaves (up to 237 mg/100 g dry weight) and Sena *et al*. (1998) reported values around 120 mg/100 g dry weight in *Hibiscus* spp. and *Ceratotheca sesamoides* leaves. This means that, despite the inorganic nature of this nutrient, this amount represents a considerable contribution to the daily dietary iron recommendations.

As previously indicated, different species should be regarded as interesting alternatives to improve the mineral intake in these populations (Freiberger *et al*. 1998; Sena *et al*. 1998). For example, *Amaranthus* spp. have shown high levels of Ca, Mg, Fe, Zn, and Mn, according to different studies (FAO 1990; Kruger *et al*. 1998; Nesamvuni *et al*. 2001; Odhav *et al*. 2007). Also *Hibiscus trionum*, analyzed in a study conducted in South Africa by Steyn *et al*. (2001), is remarkable for its high levels of Ca, Mg, P, and Zn. The encouragement of the inclusion of these indigenous species in the diet of African populations could possibly ameliorate some of their nutritional problems.

Vitamins are one of the most relevant contributions of vegetables to the diet. Due to the high water content and low lipid amount, hydrosoluble vitamins are more important in vegetables than liposoluble ones. Leafy vegetables are well known as good folate sources, and an increase in their consumption would be a good strategy to avoid the consequences of folate deficiencies, mainly defects in neural tube formation (spina bifida or anencephaly), among other disorders (Kondo *et al*. 2005). To avoid these diseases, a daily intake of 200–400 μg/day, mainly in the preconception period, is recommended; in all cases an additional 100–200 μg/day should be ingested during pregnancy (especially in the first months, to reduce the risk of neural tube formation defects) and lactation, according to different national and international recommendations recorded by Cuervo *et al*. (2009). In this respect, African wild leafy vegetables such as *Bidens pilosa* or *Cleome* spp. provide more than 300 μg folate/100 g of

fresh plant (see Table 7.1). Both are abundant weeds, very widely used across Africa; *B. pilosa* (blackjack) leaves are very popular as a pot-herb (although certain authors regard it as an irritant), and *Cleome gynandra* L. is eaten regularly in Malawi, while in Kenya it is reserved for special occasions and ceremonies, as well as for women in labor. The folate content of both species is in the range of or even higher than many cultivated vegetables (Souci *et al*. 2008), making them interesting options whose consumption should be encouraged, especially for African women.

As for *C. gynandra* or *C. monophylla* L., these plants have shown good nutritional potential as sources of folate but also for their vitamin C content. Vitamin C deficiency provokes scurvy, whose primary symptoms are haemorrhage in the gums, skin, bones, and joints, and the failure of wound healing. Fresh fruits and vegetables are the best sources of vitamin C, and wild edible species are no exception. Many wild leafy vegetables of *Amaranthus* and *Brassica* spp. are remarkable for containing more than 100 mg of ascorbic acid/100 g fresh plant, reaching almost 200 mg/100 g in *Vernonia* spp.

Dietary recommendations for vitamin C are established with a wide range of variation (45–120 mg/day for adults); however, many of these species can provide the whole daily requirement in one 100 g portion. A limitation of this contribution is the fact that vitamin C is a very heat-labile compound so very variable losses of this vitamin may occur, depending on the way of cooking vegetables, from 14% to 95% (Morales 2012; Somsub *et al*. 2008; Yadav *et al*. 1997). In this way, raw consumption of the plants is recommended if possible, and if the plant has to be processed, pressure or steam cooking, when available, is usually preferable to traditional methods to minimize these losses.

Regarding liposoluble vitamins, reference should be made to vitamin A, which is present in food plants not as retinol but as provitamin A carotenoids (α-carotene, β-carotene, and β-cryptoxanthin), which are biotransformed to retinol in the human body (Britton *et al*. 1995; Ibrahim *et al*. 1991; Patton *et al*. 1990). Their vitamin A activity is measured as retinol equivalents (RE). Besides this, most carotenoid compounds play an important role as dietary antioxidants. Additionally, lutein and zeaxanthin may be

protective for eye disease because they absorb damaging blue light that enters the eye (Krinsky & Johnson 2005). Other nonprovitamin A carotenoids important in plant tissues include neoxanthin and violaxanthin (frequent in leafy vegetables) and lycopene (present in some fruits).

Food sources of these compounds include a variety of fruits and vegetables. Some authors have measured provitamin A activity in some African wild edible plants from their carotenoid contents. Not many studies include a pormenorized analysis of carotenoids in African wild vegetables; however, Tlili *et al*. (2009) reported lutein and β-carotene as major carotenoids in Tunisian leafy vegetables, particularly in *Capparis spinosa* L. edible parts, at levels of 1234 mg/100 g and 234 mg/100 g respectively, while other carotenoids were present in much lower amounts.

As previously mentioned, deficiency of vitamin A is a major cause of premature death in developing countries, particularly among children, and manifests with xerophthalmia, night blindness, poor reproductive health, increased risk of anemia, and slowed growth and development (FAO/WHO 2004). The daily intake of vitamin A for adults should range between 0.5 and 1 mg RE/day to avoid these problems (Cuervo *et al*. 2009). Among African wild edible plants, again *B. pilosa* stands out as a good source of vitamin A, reaching almost 1 mg RE, which means that a 100 g portion can provide the total daily requirement for an adult; other leafy vegetables are also good alternatives to improve vitamin A status, as can be seen in Table 7.1. This is the case for *Ipomoea batatas* L. (sweet potato) and *Manihot esculenta* Crantz (cassava), both of them more widely known for the use of their tubers but whose leaves are also eaten in many tropical areas of Africa and Asia. The leaves of sweet potato may reach up to 1 mg RE/100 g. Cassava leaves have been reported to provide almost 2 mg RE/100 g, while the leaves of different *Cleome* and *Corchorus* aspecies (both of them very popular in different part of Africa) have shown high RE levels. Also, *Momordica foetida* Schumach (bitter melon) leaves contain almost 1 mg RE/100 g; the fruits of *Momordica* species are also eaten (some varieties are bitter, due to the presence of momordicoside, a special type ofcucurbitacin), usually removed by soaking in salt water, boiling or frying (Gry *et*

al. 2006).

These findings are of great importance in areas where half the population is suffering from vitamin A deficiency, since these autochthonous plants could be easily gathered or adapted to cultivation with great nutritional benefit, especially for children and other at-risk groups. Many authors have reported on the cultivation of wild African edible species, such as *Amaranthus* spp. or *Corchorus* spp. (Aju *et al*. 2013; FAO 1988; Mathowa *et al*. 2014).

Other bioactive compounds in African wild vegetables include dietary fiber and phenolic compounds. Fiber has been measured in several wild plant foods and many of them have shown more than 3 g/100 g fw, the level commonly used as a minimum to indicate that a food is rich in fiber (European Parliament and Council 2006); some plants even contain more than 6 g/100 g fw, as in the case of *Urtica urens* L. and *Euphorbia hirta* L. edible parts (6.7 and 7.7 g/100 g fw, respectively); this information can be seen in Table 7.2. These species could contribute to the dietary fiber intake in African populations, improving their gastrointestinal health, as well as other effects of dietary fiber, such as those related to regulatory activity of the immune system (Brett & Waldron 1996).

Table 7.2 Leafy vegetables traditionally consumed in Africa, standing out as sources of bioactive compounds. Data are given per 100 g of fresh weight.

Species	Dietary fiber (g/100 g)	Carotenoids/ phenolic compounds/ tocopherols	References
Adansonia digitata L.	3	–	FAO 1990
Amaranthus spp.	3–3.3	Carotenoids (88–194 mg/100 g dw); total phenols (1057–2181 mg GAE/100 g	Nesamvuni *et al*. 2001; Odhav *et al*. 2007

		dw)	
Bidens pilosa L.	3–6	–	FAO 1990, Kruger *et al*. 1998; Nesamvuni *et al*. 2001, Odhav *et al*. 2007
Capparis spinosa L.	–	Lutein (234 mg/100 g); β-carotene (104 mg/100 g); neoxanthin (4.55 mg/100 g); violaxanthin (1.77 mg/100 g); α-tocopherol (20.2 mg/100 g)	Tlili *et al*. 2009
Chenopodium album L.	–	Total phenols (8.61 mg TAE/g extract); proanthocyanidins (3.8 mg CE/g extract)	Afolayan & Jimoh 2009
Cleome spp. (*C. gynandra* L., *C. monophylla* L.)	2.7–4.5	–	Nesamvuni *et al*. 2001
Euphorbia hirta L.	7.7	Tannins (1222 mg/100 g)	Wallace *et al*. 1998
Ipomoea involucrata Hance	–	Tannins (869 mg /100 g)	Wallace *et al*. 1998
Lesianthera	4	–	Isong & Idiong

africana L.			1997
Manihot esculenta L.	4	–	Odhav *et al.* 2007
Momordica foetida L.	3–3.15	–	FAO 1990, Nesamvuni *et al.* 2001
Moringa peregrina Fiori		Tocopherols (28 mg/100 g)	Yang *et al.* 2006
Opuntia microdasys (Lehm.) Pfeiff.	5.4	Tocopherols (6.9 mg/100 g dw); hexosyl ferulic acid (852 µg/g extract); isorhamnetin O-(rhamnosyl)-rutinoside (2507 µg/g extract)	Chahdoura *et al.* 2014
Opuntia macrorhiza (Engelm.)	6.2	Tocopherols (5.1 mg/100 g dw); piscidic acid (3400 µg/g extract); eucomic acid (1688 µg/g extract)	Chahdoura *et al.* 2014
Senna occidentalis L.	3	–	Odhav *et al.* 2007
Sonchus asper L.	–	Total phenols (10.5 mg TAE/g extract); proanthocyanidins (2.2 mg CE/g extract)	Afolayan & Jimoh 2009
Spinacia	3	–	Kruger *et al.*

oleracea L.			1998
Urtica urens L.	6.9	Total phenols (6.7 mg TAE/g extract); Proanthocayanidins (3.9 mg CE/g extract)	Afolayan & Jimoh 2009
Vigna unguiculata L.	4	–	Kruger *et al.* 1998
Xanthosomas spp.	–	Tannins (655 mg/100 g)	Wallace *et al.* 1998

CE, catechin equivalent; dw, dry weight; GAE, gallic acid equivalent; TAE, tannic acid equivalent.

Euphorbia hirta also stands out as a good source of phenolic compounds, with more than 1 g of tannins per 100 g of fresh plant (Wallace *et al.* 1998); this is probably related to high antioxidant activity in this species. In vegetables, often tannins are bound to fiber polymers and remain undigested in the gut, acting as antioxidants at this level. In this case, the presence of a high amount of fiber and tannins together suggests a very interesting effect of this plant at gastrointestinal level, so it would be a very good choice for food. Many of the reports about phenolic content in wild African plants are focused on medicinal plants (Boulanouar *et al.* 2013; Djeridane *et al.* 2007) rather than food plants, searching for biological/pharmacological activities of these compounds as antimicrobial, antioxidant or antiinflammatory agents. For example, the aqueous extract of *Urtica urens* has shown antimicrobial activity against several Gram-positive and Gram-negative microorganisms, comparable to some antibiotics (Jimoh *et al.* 2010), and *U. dioica* L. has shown great potential for the treatment of urinary pathologies (Zhang *et al.* 2014). Lindsey *et al.* (2002) studied wild food plants growing widely in South Africa, finding that extracts from greens such as *Sisymbrium thellungii* O. E. Schulz, *Hypoxis hemerocallidea* Fisch., C. A. Mey. & Avé-Lall, and *U. dioica* showed interesting properties for the inhibition of lipid peroxidation. More studies would be desirable to determine the pormenorized composition of

phenolics or other compounds responsible for these actions in African wild vegetables, which could help to improve the antioxidant potential of African diets.

In many African countries, different food-based strategies, driven by nongovernmental organizations and other local institutions, have already met with good results and acceptance (Oniang'o *et al*. 2008; Smith & Eyzaguirre 2007), in the move to improve the nutritional quality of African diets.

7.2.2 Wild Vegetables Consumed in the Americas

The American continent is a good example of economic, social, and cultural differences, from the countries in the north, with a high degree of economic development, to Central and South America, where areas with a better economic status are mixed with more depressed areas. Many factors have contributed to this map, including historical, demographic, and political reasons, among others. Due to the size and geographical characteristics of the continent, almost all the different types of climates can be found, from polar to tropical, which enormously influences the vegetation and human relationship with the environment (Kottek *et al*. 2006).

The tribes of ancient inhabitants across the whole continent were very well adapted to the natural world. According to the first chronicles of European explorers, they interacted every day with the native plants and animals, transforming plants, animals, and soil materials into food, medicines, and utensils for daily life. Many tribes were nomads, and this made it possible to gather plants from different places, not randomly but with a clear objective of protecting wild animal and plant populations, as a part of their own lives, preserving spectacular landscapes, such as prairies, forests, grasslands, and savannahs, and achieving an intimacy with wildlife unmatched by any of the modern trends of returning to nature (Anderson 2005; Barrera *et al*. 1977).

Later, European colonization contributed to great changes in landscape, agricultural practices, society, and food habits, and subsequent evolution has brought about the loss of a great part of

the cultural heritage of indigenous people (Anderson 2005; Stoffle *et al*. 1990). Nowadays, in the most developed countries, gathering of wild plants has been replaced by use of cultivated foods and intensive agriculture practices, and very few of these ancient traditions exist, linked to small areas of indigenous population remaining in reserves or similar places. In other countries, where indigenous characteristics remain in many aspects of life and traditions, the presence of wild vegetables in the diet has been better preserved, and many wild plants can be found in the gastronomy of these countries, gathered from the wild, sold in local markets or cultivated in house gardens, as a part of sustainable development of many rural communities (Herrera Molina *et al*. 2014a).

The American continent presents huge social differences, making it possible to find the two extremes of malnutrition: the type linked to abundance of food (overweight, obesity, diabetes, cardiovascular disease or metabolic syndrome), often accompanied by subclinical deficiencies of some vitamins and minerals caused by the lack of fresh fruit and vegetable intake, and the type suffering the consequences of undernutrition. According to WHO data (Benoist *et al*. 2008), 29.3% of preschool-aged children (<5 years old), 24.1% of pregnant women, and 17.8% of nonpregnant women of fertile age are affected by anemia. The prevalence of vitamin A deficiency in America is around 15.6%, with more than 8 million preschool children suffering from biochemical vitamin A deficiency and low levels of serum retinol (WHO 2009). In rural communities eating a wide variety of fresh fruits and vegetables which provide vitamins, minerals, fiber, and other bioactive compounds, health status is usually good, and the displacement of autochthonous foods by the introduction of less healthy habits (often linked to consumption of modern diets) should be avoided for cultural and nutritional reasons.

Tables 7.3 and 7.4 present data about wild plant nutrients and bioactive compounds. Some vegetables, such as *Allium vineales* L., *Glechoma hederacea* L. or *Plantago major* leaves, can be highlighted for their high contribution of vitamin A (10 000–19 000 IU per 100 g, around 0.5–1 mg RE/100 g); they could be a tool to improve the vitamin status of American populations.

Vitamin C is abundant in the leaves of *Alliaria officinalis* L. and *Allium* species, with values of more than 80 mg/100 g, higher than many cultivated foods generally considered as vitamin C sources (e.g. citric fruits). The culinary processing of these vegetables should be reduced to the minimum to preserve their vitamin C content. Very few data about the folate content of American wild vegetables are available in the literature: low levels have been reported, for example, in *Urtica dioica*, but as for other wild leafy vegetables eaten all over the world, many of them may have levels to improve the nutritional status of the population, especially for women of fertile age, avoiding fetal malformations linked to deficiencies of folic acid. More research should be done in this field with the purpose of establishing recommendations that could be easy to follow since in Central and South America there are many populations using wild plants in their habitual diet.

Table 7.3 Vegetables traditionally consumed in America, standing out as sources of vitamins or minerals. Data are given per 100 g of fresh weight.

Species	Edible part	Vitamins	Minerals	References
North America				
Achillea millefolium L.	Leaves	–	K (645 mg/100 g); Ca (225 mg/100 g); Mg (53 mg/100 g); Fe (13.1 mg/100 g); Cu (0.2 mg/100 g); Zn (0.7 mg/100 g); Mn (4.0 mg/100 g)	Kuhnlein & Turner 1991
Alliaria officinalis	Leaves	Vitamin C (190	–	Zennie *et al.* 1977

L.		mg/100 g)		
Allium vineales L.	Leaves	Vitamin C (130 mg/100 g)	–	Zennie *et al*. 1977
Allium tricoccum A.T.	Leaves	Vitamin C (80 mg/100 g)	–	Zennie *et al*. 1977
Amaranthus palmeri S.Watson	Leaves	RE (385 mg/100 g); vitamin B_2 (240 µg/100 g); B_3 (1.2 mg/100 g); C (72.5 mg/100 g)	K (411 mg/100 g); Ca (362 mg/100 g); Fe (4.5 mg/100 g)	Kuhnlein & Turner 1991
Amaranthus spp.	Leaves	RE (292 mg/100 g); vitamin B_2 (160 µg/100 g); B_3 (1.4 mg/100 g); C (43.3 mg/100 g)	K (611 mg/100 g); Ca (267 mg/100 g); MG (55 mg/100 g); Cu (0.2 mg/100 g); Fe (3.9 mg/100 g); Zn (0.9 mg/100 g)	Kuhnlein & Turner 1991
Chenopodium album L.	Leaves	Vitamin B_3 (120 µg/100 g); C (70 mg/100 g); K (347 µg/100 g)	Ca (246 mg/100 g); Fe (1.8 mg/100 g); Cu (2.3 mg/100 g); Zn (2.3 mg/100 g).	Kuhnlein 1990
Chenopodium	Leaves	Vitamin B_2	Ca (304	Kuhnlein &

ambrosioides L.		(280 μg/100 g); B_3 (800 μg/100 g); C (11 mg/100 g)	mg/100 g); Fe (5.2 mg/100 g)	Turner 1991
Cichorium intybus L.	Leaves	RE (400 mg/100 g); vitamin B_3 (500 μg/100 g)	K (420 mg/100 g); Fe (0.9 mg/100 g)	Kuhnlein & Turner 1991
Duschenea indica (=*Potentilla indica* (Andrews) T. Wolf)	Leaves	Vitamin C (79 mg/100 g)	–	Zennie *et al*. 1977
Epilobium angustifolium L.	Young stems	–	Cu (0.7 μg/100 g)	Kuhnlein 1990
Glechoma hederacea L.	Leaves	Vitamin C (44 mg/100 g)	–	Zennie *et al*. 1977
Malva parviflora L.	Leaves	Vitamin C (65 mg/100 g)	Ca (324 mg/100 g)	Kuhnlein & Turner 1991
Mentha spicata L.	Leaves	RE (856 mg/100 g); vitamin B_3 (0.4 mg/100 g); C (68 mg/100 g)	Ca (200 mg/100 g); Fe (15.6 mg/100 g)	Kuhnlein & Turner 1991
Oxalis stricta L.	Leaves	Vitamin C (59–79 mg/100 g)	–	Zennie *et al*. 1977
Plantago	Leaves	RE (252	K (277	Kuhnlein &

major L.		mg/100 g); vitamin B_2 (0.28 mg/100 g); B (0.8 mg/100 g), C (8–19 mg/100 g)	mg/100 g); Ca (184 mg/100 g)	Turner 1991 Zennie *et al.* 1977;
Porphyra perforata J. Ag.	Leaves	–	Ca (230 mg/100 g); Mg (623 mg/100 g); Fe (2.9 mg/100 g); Cu (1.7 mg/100 g)	Kuhnlein 1990
Rumex acetosella L.	Young leaves	Vitamin B_3 (430 µg/100 g); C (33.5 mg/100 g)	Fe (2.3 mg/100 g); Cu (1.2 mg/100 g); Zn (1.2 mg/100 g)	Kuhnlein 1990
Urtica dioica L.	–	RE (2248 mg/100 g); vitamin B_2 (160–220 µg/100 g); B_3 (300–371 µg/100 g); C (75 mg/100 g)	Ca (236–452 mg/100 g); Mg (63–235 mg/100 g); Fe (1–1.26 mg/100 g); Zn (1.9–2 mg/100 g)	Kuhnlein & Turner 1991; Phillips *et al.* 2014
Central America				
Agave shrevei Gentry sbsp.	Basal leaves	–	Ca (1061 mg/100 g); Mg (382 mg/100 g);	Laferriere *et al.* 1991

matapensis			Fe (5.6 mg/100 g); Zn (1.9 mg/100 g)	
Hedeoma patens M.E. Jones	Shoots	–	Ca (1253 mg/100 g); Mg (99.1 mg/100 g); Cu (0.8 mg/100 g); Zn (2.8 mg/100 g)	Laferriere *et al*. 1991
Monarda austromontana Epling	Shoots	–	Ca (1303 mg/100 g); Mg (431.3 mg/100 g); Cu (1.2 mg/100 g); Zn (4.9 mg/100 g)	Laferriere *et al*. 1991
Opuntia spp.	Cladodes	Vitamin B_1 (140 μg/100 g); B_2 (60–80 μg/100 g); B_3 (46–240 μg/100 g); C (7–22 mg/100 g)	Ca (12.8–81 mg/100 g); Mg (16.1–98.4 mg/100 g); Fe (0.4–2.34 mg/100 g)	Feugang *et al*. 2015; Rosquero Perez 2001
Opuntia durangensis Brilton & Rose	Cladodes	–	Ca (2422 mg/100 g); Mg (1938 mg/100 g); Fe (9.6 mg/100 g); Cu (0.2 mg/100 g)	Laferriere *et al*. 1991

Opuntia macrorhiza Engelm.	Cladodes	–	Ca (1541 mg/100 g); Mg (917 mg/100 g); Fe (8.5 mg/100 g); Cu (1.3 µg/100 g); Zn (5.6 mg/100 g)	Laferriere *et al*. 1991
Opuntia robusta Pfeiff.	Cladodes	–	Ca (1235 mg/100 g); Mg (839 mg/100 g); Fe (15.6 mg/100 g); Cu (0.5 mg/100 g); Zn (16.1 mg/100 g)	Laferriere *et al*. 1991
Teloxys ambrosioides (L.) W.A. Weber	Shoots	–	Ca (1892 mg/100 g); Mg (1418 mg/100 g); Fe (12.7 mg/100 g); Cu (0.9 mg/100 g)	Laferriere *et al*. 1991
South America				
Trichanthera gigantea Humb. & Bonpl. ex Steud.	Leaves	–	Ca (972–1242 mg/100 g); Mg (153–202 mg/100 g); Fe (2.15–8.42	Leterme *et al*. 2006

			mg/100 g); Cu (0.21–0.44 mg/100 g);	
Xanthosoma spp.	Leaves	–	Ca (280–372 mg/100 g); Mg (53–104 mg/100 g); Fe (0.66–6.42 mg/100 g); Cu (0.08–0.28 mg/100 g)	Leterme *et al*. 2006

IU of vitamin A are obtained by multiplying the amount of β-carotene (μg) by a factor of 1.6.

Table 7.4 Vegetables traditionally consumed in America standing out as sources of bioactive compounds. Data are given per 100 g of fresh weight.

Species	**Edible part**	**Dietary fiber (g/100 g)**	**Carotenoids/ fatty acids (relative percentage)/ phenolics**	**References**
North America				
Chamerion angustifolium (L.) Holub	Aerial part	–	C18:2 (10.2–17.4%); C18:3 (35.9–40.3%)	Malainey *et al*. 1999
Chenopodium album L.	Young leaves	1.5	β- carotene (640 μg/100 g) C18:2	Kuhnlein 1990; Malainey *et al*. 1999

			(12.8–13.9%); C18:3 (34.1–35.7%)	
Urtica spp.	Aerial part	–	C18:2 (18.7%); C18:3 (30.8%)	Malainey *et al*. 1999
Conyza canadiensis	Aerial part	–	C18:2 (21.3%)	Malainey *et al*. 1999
Heracleum mantegazzianum Sommier & Levier	Aerial part	–	C18:2 (20.8–27.6%); C18:3 (16.1–22.9%)	Malainey *et al*. 1999
Lycopus americanus Muhl.	Aerial part	–	C18:2 (10.35%); C18:3 (30.71%)	Malainey *et al*. 1999
Maianthemum racemosum L.	Aerial part	–	C18:2 (21.3–29.2%); C18:3 (27.3–37.6%)	Malainey *et al*. 1999
Rumex spp.	Aerial part	–	C18:2 (11.6–16.4%); C18:3 (31.3–43.9%)	Malainey *et al*. 1999
Rumex acetosella L.	Young leaves	1.1	–	Kuhnlein 1990
Rumex	Leaves	–	–	Chirinos *et*

crispus L.				*al*. 2013
Rubus parviflorus Nutt.	Young stems	1.0	–	Kuhnlein 1990
Smilax ornata Lem.	Aerial part	–	C18:2 (6.38–16.3%); C18:3 (33.1–49.9%)	Malainey *et al*. 1999
Solidago canadensis L.	Aerial part	–	C18:2 (10.5–18.1%); C18:3 (30.1–42.5%)	Malainey *et al*. 1999
Stellaria porsildii C.C. Chinnappa	Aerial part	–	C18:2 (31.9%); C18:3 (15.1%)	Malainey *et al*. 1999
Urtica dioica L.	Aerial part	4.8	–	Phillips *et al*. 2014
Central America				
Agave shrevei Gentry sbsp. *matapensis*	Basal leaves	21.7	–	Laferriere *et al*. 1991
Opuntia spp.	Cladodes	1–10.3	β-carotene (2.25–53.5 mg/100 g)	Feugang *et al*. 2015; Rosquero Pérez 2001
Opuntia ficus-indica (L.) Mill.	Cladodes	7.7 8.94	Isoquercetin (267–396.7 µg/g dw); isorharmnetin,	Granda Neri 2004' Guevara-Figueroa *et*

			3, o-glucoside (127.6–322.1 μg/g dw); nicotiflorin (317.7–1173.0 μg/g dw); rutin (147.0–261.7 μg/g dw); narcissin (567.1–1371 μg/g dw)	*al*. 2010
Opuntia leucotricha DC.	Cladodes	7.1	Isoquercetin (50.0 μg/g dw); isorharmnetin, 3, o-glucoside (45.9 μg/g dw); narcissin (220.1 μg/g dw)	Guevara-Figueroa *et al*. 2010
Opuntia robusta Pfeiff.	Cladodes	17.43	Isoquercetin (106.0 μg/g dw); isorharmnetin, 3, O-glucoside (99.3 μg/g dw); nicotiflorin (910.7 μg/g dw); rutin	Guevara-Figueroa *et al*. 2010; Laferriere *et al*. 1991

			(140.1 µg/g dw)	
Opuntia durangensis	Cladode	16.33	–	Laferriere *et al*. 1991
Opuntia macrorhiza Engelm.	Cladode	7.77	–	Laferriere *et al*. 1991
Opuntia lindheimeri Engelm.	Cladodes	3.02	–	Granda Neri 2004
Opuntia imbricada (Haw.) DC.	Cladodes	11.5	–	Granda Neri 2004
Opuntia lindheimeri var. *tricolor*	Cladodes	10.7–11.4	–	Granda Neri 2004
South America				
Agave americana L.	Leaves	–	Total phenolics: 9.8–10 (mg GAE/g dw); flavonoids: 3.0–3.1 (mg QE*/g dw)	Chirino *et al*. 2013
Alnus acuminata Kunth.	Leaves	–	Total phenolics: 71.2–73.4 (mg GAE/g dw); flavonoids: 13.11–13.63 (mg QE*/g dw), 0.28–0.30 (mg CE**/g dw)	Chirino *et al*. 2013

Amaranthus viridis L.	Leaves	–	Total carotenoids (0.347–0.468 mg/100 g)	Mercadante & Rodriguez-Amaya 1990
Cassia hookeriana Gillies ex Hook.	Leaves	–	Total phenolics: 18.6–18.8 (mg GAE/g dw); flavonoids: 13.22–13.86 (mg QE*/g dw), 0.08 (mg CE**/g dw)	Chirino *et al*. 2013
Cestrum auriculatum L'Hér.	Leaves	–	Total phenolics: 6.4–6.6 (mg GAE/g dw); flavonoids: 2.32–2.34 (mg QE*/g dw)	Chirino *et al*. 2013
Clinopodium bolivianum Kuntze	Leaves	–	Total phenolics: 56.4–57.2 (mg GAE/g dw); flavonoids: 9.6–9.7(mg QE*/g dw); 1.14–1.18 (mg CE**/g dw)	Chirino *et al*. 2013
Jungia paniculata	Leaves	–	Total phenolics:	Chirino *et al*. 2013

A. Gray				28.0–28.4 (mg GAE/g dw); flavonoids: 25.2–25.3(mg QE*/g dw)	
Lepidium pseudodidymum Thell. in Druce	Leaves	–		Total carotenoids (0.237–0.280 mg/100 g)	Mercadante & Rodriguez-Amaya 1990
Lepechinia meyenii Epling	Leaves	–		Total phenolics: 60.5–61.9 (mg GAE/g dw); flavonoids: 7.7–8.2 (mg QE*/g dw)	Chirino *et al*. 2013
Melissa officinalis L.	Leaves	–		Total phenolics: 30.7–31.7 (mg GAE/g dw); flavonoids: 4.0–4.2 (mg QE*/g dw)	Chirino *et al*. 2013
Mutisia acuminata Ruiz & Pav.	Leaves	–		Total phenolics: 58.5–60.3 (mg GAE/g dw); flavonoids: 6.59–6.85 (mg QE*/g dw)	Chirino *et al*. 2013

Portulaca oleracea L.	Leaves	–	Total carotenoids (0.071–0.109 mg/100 g)	Mercadante & Rodriguez-Amaya 1990
Sonchus oleraceus L.	Leaves	–	Total carotenoids (0.225–0.361 mg/100 g)	Mercadante & Rodriguez-Amaya 1990
Xanthosoma spp.	Leaves	–	Total carotenoids (0.149–0.334 mg/100 g)	Mercadante & Rodriguez-Amaya 1990
Oenothera rosea Aiton.	Leaves	–	Total phenolics: 64.5–65.9 (mg GAE/g dw); flavonoids: 26.6–26.8 (mgQE*/g dw)	Chirino *et al*. 2013
Schimus molle L.	Leaves	–	Total phenolics: 52.1–53.3 (mg GAE/g dw); flavonoids: 11.0–11.2 (mg QE*/g dw); 8.4–8.6 (mg CE**/g dw)	Chirino *et al*. 2013

* Content of flavan-3-ols, flavan-4-ols, flavan-3,4-diols, flavanones, and derivatives;

** content of flavones and flavonols.

CE, catechin equivalent; dw, dry weight; GAE, gallic acid equivalent; QE, quercetin equivalent.

With regard to minerals, calcium has been found in high levels in the leaves of *Chenopodium album* L. and *Urtica dioica* (230–452 mg/100 g), reported as traditionally eaten in North America, as well as in other Central and South America species (*Hedeoma patens* M. E. Jones, *Monarda citriodora* Cerv. ex Lag. *var. austromontana* (Epling) B.L.Turner, *Teloxys ambrosioides* (L.) W. A. Weber, *Trichanthera gigantea* Humb. & Bonpl. ex Steud. and *Xanthosoma* spp.), most with levels of more than 1 g/100 g). Special attention should go to the cactus species, very popular in Central and South America, such as *Agave shrevi* Gentry, and cladodes of many *Opuntia* species, which represent a very important staple food in Mexico (even present in the arms of the country), as either wild or cultivated species. Cladodes are important calcium sources (1–2.5 mg/100 g), and the oxalate content has also been reported in some studies on cultivated *Opuntia ficus-indica* L. Miller (over 1 g/100 g). Moreover, the ratio of oxalic acid/Ca is often favorable to absorption and *in vitro* studies about Ca bioaccessibility in cultivated *Opuntia* cladodes (Ramírez Moreno *et al*. 2011) have shown 15–50% of gut-accessible Ca. Given the high Ca content of these vegetables, this could mean more than 150 mg/100 g of accessible Ca (even taking into account the presence of oxalates), which surpasses the daily recommendation for this mineral. For that reason, and due to its strong presence in American diets, cladodes are a valuable vegetable, except for those suffering from renal problems. As can be seen in Tables 7.3 and 7.4, *Opuntia* cladodes also provide high levels of Mg, Fe, and dietary fiber; this is also seen in other cacti such as *Agave shrevi*.

Other bioactive compounds of interest provided by traditional American plants are phenolic compounds, where flavonoids usually represent an interesting fraction (see Table 7.4), and polyunsaturated fatty acids such as α-linolenic (C18:3n3) and linoleic (C18:2n6) acids, being the major ones in most leafy vegetables. All these compounds make these plants useful tools to improve the health status of American populations, with the added

value of using their own natural resources, and for these reasons their consumption should be preserved and valued.

7.2.3 Asian Wild Edible Greens

The largest and most diverse continent in the world is Asia, with the highest and the lowest points on the surface of the Earth, the longest coastline of any continent, and wide environmental. Consequently, it produces the most varied forms of vegetation and animal life on Earth. Using the Köppen climatic classification, Asia may be divided into three major climatic regions: Siberia (north-east Asia), Monsoon (south-east Asia) and Desert (west and central Asia) (Dando 2005).

Asia is a continent of contrasts – it includes developed and rich countries as Japan, but also many poor areas in developing tropical countries such as India, China, Pakistan, Iran, Thailand, etc. These countries generally have problems with food supply due to rapid population growth, shortage of land for cultivation, high prices of available staples and restrictions on the importation of food. This has resulted in a high incidence of hunger and people suffering from malnutrition (Seal 2011). Vitamin A deficiency and age-related macular degeneration are accepted as serious public health problems among children and adults in India. It is reported that 25% of the 15 million blind in the world are from India (WHO 2000). It is known that vitamin A deficiency and age-related macular degeneration are primarily due to inadequacy of provitamin A and macular pigments in the diet (Raju *et al*. 2007). Also, iron deficiency is a public health problem in developing countries because the staple foods consist mainly of rice, cereal, grains, and vegetables more than animal products (Nutrition Formulation 1982).

Since traditional medicinal plants and food are believed to share a common origin in Chinese tradition, it is very difficult to distinguish between the two. In fact, many medicinal plants have been used as flavors, pigments, and foods (Li *et al*. 2013). Due to the economic situation in Pakistan, India and other developing

countries, the main components of the diet of the diverse ethnic groups are wild plants (Imran *et al*. 2009; Sundriyal & Sundriyal 2004). Nevertheless, there is still an enormous amount of plant material which has not been studied and whose nutritional composition is unknown.

Previously conducted ethnobotanical studies (Bandyopadhyay & Mukherjee 2009; Cruz-Garcia & Price 2011; Kang *et al*. 2013) detail the main wild edible Asian greens discussed in this chapter. *Momordica dioica* Roxb., *Portulaca oleracea*, *Centella asiatica*, *Commelina benghalensis* L., *Amaranthus* spp., *Chenopodium album*, *Urtica dioica*, *Ipomoea* spp., *Rumex* spp., *Dioscorea* spp., *and Diplazium esculentum* (Retz) Sw. are widely spread in China, Thailand, Indonesia and many regions of the Arabian sea as Pakistan, India and Iran (Aberoumand & Deokule 2009; Anusuya *et al*. 2012; Gupta *et al*. 2005; Imran *et al*. 2009; Khattak 2011; Pradhan *et al*. 2015; Raghuvanshi *et al*. 2001; Sharifi-Rad *et al*. 2014; Sultan *et al*. 2009; Vishwakarma & Dubey 2011).

Vitamins and minerals present in 100 g of plant with a higher content than 15% of the daily recommendations of nutrients given by the FNB (Trumbo *et al*. 2002) or FAO/WHO (2004) are shown in Table 7.5. Generally, these wild greens have a high mineral content, iron being the main element. Some Asian wild edible plants could provide 100% of the daily recommendation of iron (9 mg/100 g), such as *Portulaca oleracea*, *Amaranthus* spp., *Centella asiatica*, *Sonchus arvensis* L., and *Digera arvensis* Forsk. (see Table 7.5). Public health problems related to Fe deficiency, such as anemia, could be palliated by including these wild greens in the diet of at-risk populations.

Table 7.5 Vegetables traditionally consumed in Asia, standing out as sources of vitamins or minerals. Data are given per 100 g of fresh weight.

Species	Edible part	Vitamins	Minerals	References
Amaranthus spp.	Leaves	Vitamin C (39–44 mg/100 g)	K (382–433 mg/100 g); Ca (239	Gupta *et al*. 2005; Lata *et al*. 2011;

			mg/100 g); Mg (253 mg/100 g); Fe (5.8–15 mg/100 g); Cu (1.1 mg/100 g); Cr (140 µg/100 g)	Pradhan *et al*. 2015
Bauhenia purpurea L.	Leaves	Vitamin B_1 (0.1 mg/100 g); B_3 (0.87 mg/100 g); C (173 mg/100 g)	Ca (156 mg/100 g); Fe (4.6 mg/100 g)	Raghuvanshi *et al*. 2001
Boerhaavia diffusa L.	Leaves	Vitamin C (16 mg/100 g)	Ca (330 mg/100 g); Mg (167 mg/100 g); Fe (7.8 mg/100 g)	Gupta *et al*. 2005
Brassica campestris L.	Leaves	Vitamin C (51 mg/100 g)	Mg (59 mg/100 g); Fe (5.7 mg/100 g); Cu (1.1 mg/100 g); Mn (12.1 mg/100 g); Zn (3.3 mg/100 g)	Khattak 2011
Celosia argentea L.	Leaves	Vitamin C (26 mg/100 g)	K (476 mg/100 g); Ca (188 mg/100 g); Mg (233	Gupta *et al*. 2005

			mg/100 g); Fe (13.1 mg/100 g); Cu (0.15 mg/100 g) and Cr (153 µg/100 g)	
Centella asiatica L. Urb.	Leaves	–	K (345 mg/100 g); Ca (174–208 mg/100 g); Mg (87 mg/100 g); Fe (4.2–15.9 mg/100 g); Cu (0.24 mg/100 g)	Gupta *et al*. 2005; Lata *et al*. 2011; Ogle *et al*. 2001
Chenopodium album L.	Leaves	Vitamin B_3 (0.71 mg/100 g); C (33.6–43.7 mg/100 g)	K (848 mg/100 g); Ca (155.7–265 mg/100 g); Mg (112 mg/100 g); Fe (4.7–5.4 mg/100 g); Cu (0.47–1.22 mg/100 g); Mn (0.9 mg/100 g); Zn (1.3–8.4 mg/100 g)	Katach *et al*. 2011; Lata *et al*. 2011; Pradhan *et al*. 2015; Raghuvanshi *et al*. 2001; Sultan *et al*. 2009
Cicer arietinum	Leaves and young	Vitamin C (105	Mg (140 mg/100 g);	Khattak 2011

L.	shoots	mg/100 g)	Fe (8.4 mg/100 g); Cu (1.5 mg/100 g); Mn (8.5 mg/100 g); Zn (3.5 mg/100 g)	
Clerodendrum colebrookianum Walp.	Leaves	–	Ca (857 mg/100 g); Fe (69.7 mg/100 g); Cu (0.76 mg/100 g); Mn (8.6 mg/100 g); Zn (8.3 mg/100 g)	Seal *et al.* 2011
Cocculus hirsutus (L.) Diels.	Leaves	Vitamin C (28 mg/100 g); B_1 (0.19 mg/100 g)	K (343 mg/100 g); Mg (35 mg/100 g); Fe (9.9 mg/100 g); Cu (0.22 mg/100 g)	Gupta *et al.* 2005
Coleus aromaticus Benth.	Leaves	–	Ca (158 mg/100 g); Mg (88 mg/100 g); Fe (2.62 mg/100 g)	Gupta *et al.* 2005
Commelina benghalensis L.	Leaves	Vitamin C (46 mg/100 g)	K (473 mg/100 g); Mg (77 mg/100 g); Fe (2.5–7.1	Gupta *et al.* 2005; Lata *et al.* 2011

			mg/100 g); Cr (115 µg/100 g)	
Cucurbita maxima	Leaves	Vitamin B_1 (0.20 mg/100 g); C (37 mg/100 g)	K (368 mg/100 g); Ca (302 mg/100 g); Mg (150 mg/100 g); Fe (4.4 mg/100 g); Cu (0.19 mg/100 g); Cr (49 µg/100 g)	Gupta *et al.* 2005
Delonix elata (L.) Gamble	Leaves	Vitamin B_1 (0.33 mg/100 g); C (295 mg/100 g)	K (365 mg/100 g); Mg (59 mg/100 g); Fe (6.2 mg/100 g); Cu (0.27 mg/100 g); Cr (68 µg/100 g)	Gupta *et al.* 2005
Digera arvensis Forssk.	Leaves	Vitamin C (49 mg/100 g)	K (604 mg/100 g); Ca (506 mg/100 g); Mg (232 mg/100 g); Fe (17.7 mg/100 g); Cu (0.16 mg/100 g)	Gupta *et al.* 2005
Dioscorea spp.	Shoots	–	K (360 mg/100 g);	Shanthakumari *et al.* 2008

			Ca (191 mg/100 g); Mg (42.6–179 mg/100 g); Fe (2.7–5.2 mg/100 g); Cu (0.16–1.39 mg/100 g); Mn (0.59–0.97 mg/100 g)	
Diplazium esculentum (Retz.) Sw.	Leaves	Vitamin B_9 (630 µg/100 g); C (21.0–21.7 mg/100 g)	Ca (193 mg/100 g); Fe (11.2 mg/100 g); Cu (0.32 mg/100 g); Zn (2.7 mg/100 g)	Irawan *et al*. 2006; Pradhan *et al*. 2015
Fagopyrum esculentum Moench.	Leaves	Vitamin B_2 (0.24 mg/100 g); C (84.9 mg/100 g)	Ca (355 mg/100 g); Fe (6.2 mg/100 g)	Raghuvanshi *et al*. 2001
Gynandropsis penthaphylla (L.) DC.	Leaves	Vitamin B_1 (0.16 mg/100 g); C (42 mg/100 g)	K (360 mg/100 g); Ca (151 mg/100 g); Mg (77 mg/100 g); Fe (4.8 mg/100 g)	Gupta *et al*. 2005
Ipomoea aquatica Forssk.	Leaves	RE (700 µg/100 g)	Fe (1.5–4.0 mg/100 g)	Lata *et al*. 2011; Ogle *et al*. 2001;
Nasturtium	Leaves	Vitamin C	Fe (7	Pradhan *et*

officinale W.T. Aiton		(13 mg/100 g)	mg/100 g); Cu (0.58 mg/100 g); Zn (2.0 mg/100 g)	*al*. 2015
Polygala erioptera DC.	Leaves	Vitamin C (85 mg/100 g)	Mg (57 mg/100 g); Fe (4.8 mg/100 g); Cu (0.15 mg/100 g)	Gupta *et al*. 2005
Portulaca oleracea L.	Leaves	Vitamin C (50.6 mg/100 g)	Mg (87 mg/100 g); Fe (4.5–29.7 mg/100 g); Cu (2.3 mg/100 g); Mn (8.6 mg/100 g); Zn (4.7 mg/100 g)	Khattak 2011; Lata *et al*. 2011; Ogle *et al*. 2001
Rumex spp.	Leaves	–	Zn (1.7 mg/100 g)	Anusuya *et al*. 2012; Sharifi-Rad *et al*. 2014
Sonchus arvensis L.	Leaves	–	Ca (918 mg/100 g); Fe (26.0 mg/100 g); Cu (0.56 mg/100 g); Mn (1 mg/100 g); Zn (8.0 mg/100 g)	Seal *et al*. 2011
Trianthema	Leaves	Vitamin C	K (317	Gupta *et al*.

portulacastrum L.		(22 mg/100 g)	mg/100 g); Mg (153 mg/100 g); Fe (4.2 mg/100 g); Mn (0.43 mg/100 g)	2005
Urtica dioica L.	Leaves	–	K (917 mg/100 g); Ca (244 mg/100 g); Fe (8.1 mg/100 g); Cu (0.43–0.67 mg/100 g); Zn (2.3 mg/100 g)	Pradhan *et al*. 2015; Sultan *et al*. 2009

RE, retinol equivalent.

Also, these Asian wild leafy vegetables have an important vitamin C content. The daily recommendation for vitamin C reported by FAO/WHO (2004) is 45 mg/100 g. *Cicer arietinum* L. leaves contain twice the daily recommendation of vitamin C for adults, and *Delonix elata* Gamble stands out for its high vitamin C content. These plants also have good fiber content (Table 7.6), *Cocculus hirsutus* L. (Diels.) having the highest levels.

Table 7.6 Vegetables traditionally consumed in Asia, standing out as sources of bioactive compounds. Data are given per 100 g of fresh weight.

Species	Edible part	Dietary fiber (g/100 g)	Carotenoids/ PUFA/ organic acids/ phenolics/ others	References

Amaranthus spp.	Leaves	4.8	β-carotene (5410 μg/100 g); total oxalates (1270 mg/100 g); soluble oxalates (690 mg/100 g); phytic acid (1.9 mg/100 g); total phenolics (9800 mg GAE/100 g)	Gupta *et al*. 2005; Pradhan *et al*. 2015
Bambusa balcooa Roxb.	Shoots	–	Organic acids (0.1 mg/100 g); lactic acid (282 mg/100 g); piruvic acid (3.7 mg/100 g); tartaric acid (173 mg/100 g); total phenolics (97.5 catechin equivalents)	Badwaik *et al*. 2014
Bauhenia purpurea L.	Leaves	5.0	β-carotene (1302	Raghuvanshi *et al*. 2001

			μg/100 g); oxalic acid (356 mg/100 g); phytic acid (35.6 mg/100 g)	
Boerhaavia diffusa L.	Leaves	7.3	β-carotene (6730 μg/100 g); total oxalates (1250 mg/100 g); soluble oxalates (420 mg/100 g); phytic acid (4.1 mg/100 g)	Gupta *et al.* 2005
Brassica campestris L.	Leaves	–	Total phenolics (18900 mg/100 g)	Khattak 2011
Celosia argentea L.	Leaves	5.1	β-carotene (4420 μg/100 g); total oxalates (920 mg/100 g); soluble oxalates (580 mg/100 g); phytic acid	Gupta *et al.* 2005

			(2.9 mg/100 g)	
Centella asiatica L. Urb.	Leaves	4.1–5.9	β-carotene (3341–3900 μg/100 g); RE (564/100 g); total oxalates (60 mg/100 g); soluble oxalates (20 mg/100 g); phytic acid (2.1 mg/100 g)	Gupta *et al.* 2005; Lata *et al.* 2011; Ogle *et al.* 2001
Chenopodium album L.	Leaves	–	β-carotene (1838 μg/100 g); oxalic acid (142 mg/100 g); phytic acid (5.1 mg/100 g); total phenolics (2916–9800 mg GAE/100 g); phenolic acids (6.98 mg/100 g)	Khattak 2011; Pradhan *et al.* 2015 ; Raghuvanshi *et al.* 2001
Cocculus hirsutus (L.) Diels.	Leaves	11.1	β-carotene (9200 μg/100 g);	Gupta *et al.* 2005

			total oxalates (230 mg/100 g); soluble oxalates (140 mg/100 g); phytic acid (2.4 mg/100 g)	
Coleus aromaticus Benth.	Leaves	–	β-carotene (1500 µg/100 g); total oxalates (50 mg/100 g); soluble oxalates (20 mg/100 g); phytic acid (0.92 mg/100 g)	Gupta *et al.* 2005
Commelina benghalensis L.	Leaves	1.5–5.4	β-carotene (3810 µg/100 g); total oxalates (390 mg/100 g); soluble oxalates (40 mg/100 g); phytic acid (4.4 mg/100 g)	Gupta *et al.* 2005; Lata *et al.* 2011
Cucurbita	Leaves	5.8	β-carotene	Gupta *et al.*

maxima Duchesne in Lam.			(2270 µg/100 g); total oxalates (200 mg/100 g); soluble oxalates (70 mg/100 g); phytic acid (9.2 mg/100 g)	2005
Delonix elata (L.) Gamble	Leaves	8.8	β-carotene (10510 µg/100 g); total oxalates (90 mg/100 g); soluble oxalates (30 mg/100 g); phytic acid (5.1 mg/100 g)	Gupta *et al.* 2005
Digera arvensis Forssk.	Leaves	4.4	β-carotene (3360 µg/100 g); total oxalates (1410 mg/100 g); soluble oxalates (570 mg/100 g); phytic acid (2.5	Gupta *et al.* 2005

			mg/100 g)	
Dioscorea spp.	Shoots	–	Total oxalates (1.9–79.8 mg/100 g); total phenolics (14.6–105.3 mg /100 g)	Mohan & Kalidass 2010; Shanthakumari *et al*. 2008
Diplazium esculentum (Retz.) Sw.	Leaves	4.8	Total phenolics (6800 mg GAE/100 g)	Irawan *et al*. 2006; Pradhan *et al*. 2015
Fagopyrum esculentum Moench.	Leaves	–	β-carotene (3020 μg/100 g); oxalic acid (316 mg/100 g); phytic acid (5.3 mg/100 g)	Raghuvanshi *et al*. 2001
Gynandropsis penthaphylla (L.) DC.	Leaves	3.3	β-carotene (5380 μg/100 g); total oxalates (20 mg/100 g); soluble oxalates (10 mg/100 g); phytic acid (13.1 mg/100 g)	Gupta *et al*. 2005
Ipomoea aquatica	Leaves	3.2	β-carotene (4164	Lata *et al*. 2011; Ogle

Forssk.			μg/100 g)	*et al*. 2001
Malcomia africana (L.) R. Br.	Leaves	–	PUFA (61.2%); C18:3n3 (38.3%); C18:2n6 (9.2%)	Imran *et al*. 2009
Mentha longifolia (L.) Huds.	Leaves	–	PUFA (33.5%); C18:3n3 (18.0%); linoleic acid (15.5%)	Imran *et al*. 2009
Nasturtium officinale W.T. Aiton	Leaves	–	Total phenolics (1800 mg GAE/100 g)	Pradhan *et al*. 2015
Polygala erioptera DC.	Leaves	6.6	β-carotene (3830 μg/100 g); total oxalates (60 mg/100 g); soluble oxalates (10 mg/100 g); phytic acid (3.4 mg/100 g)	Gupta *et al*. 2005
Portulaca oleracea L.	Leaves	5.22	Total carotenoids (2234 mg/100 g); PUFA (48.5%); C18:3n6 (30.1%);	Katach *et al*. 2011 ; Imran *et al*. 2009

			C18:2n6 (18.5%)	
Spinacia oleracea L.	Leaves	–	PUFA (52.4%); C18:3n3 (43.4%); C18:2n6 (9%)	Imran *et al.* 2009
Stellaria media (L.) Vill.	Leaves	–	PUFA (33.7%); C18:3n3 (21.4%); C18:2n6 (12.2%)	Imran *et al.* 2009
Trianthema portulacastrum L.	Leaves	–	β-carotene (4000 μg/100 g); total oxalates (1080 mg/100 g); soluble oxalates (610 mg/100 g); phytic acid (2.0 mg/100 g)	Gupta *et al.* 2005
Urtica dioica L.	Leaves	–	PUFA (47.5%); linolenic acid (45.5%); linoleic acid (15.7%); total phenolics	Imran *et al.* 2009; Pradhan *et al.* 2015

(17 600 mg GAE/100 g)

GAE, gallic acid equivalent; PUFA, polyunsaturated fatty acid.

Some plants contain linoleic, linolenic, and palmitic acid as the main fatty acids (see Table 7.6). The main carotenoid in all the plants was β-carotene (see Table 7.6). Regarding antinutrients, *Digera arvensis* presents the highest total oxalate content and *Gynandropsis penthaphylla* (L.) DC. the highest phytic acid content (13.1 mg/100 g).

These plants are a good resource of food to combat hunger and important diseases in developing countries.

7.2.4 Vegetables Traditionally Consumed in Europe

Since the nineteenth century, socioeconomic progress and public health measures in Europe have lead to increased life expectancy, mainly as result of reduced mortality in early life. Mackenbach *et al*. (2008) found that inequalities in mortality rates are smaller in some southern European countries but very large in most countries in the eastern and Baltic regions. The WHO European Region has seen remarkable health gains arising from progressive improvements in the conditions in which people are born, grow, live, and work. However, levels of health vary significantly between countries. These differences are even greater when inequities within countries, according to gender and socioeconomic position, are considered (Groenewold *et al*. 2008; WHO 2013).

Chronic diseases are the leading cause of mortality and morbidity in Europe, and research suggests that complex conditions such as cardiovascular disease, diabetes, asthma, and chronic obstructive pulmonary disease (COPD) will mean a greater burden in the future. Many of these are connected to an aging society but also to lifestyle choices such as smoking, sexual behavior, diet, and exercise, as well as genetic predispositions (Busse *et al*. 2010).

In recent decades, public health research has focused on proximate causes of health and health inequities. The European Commission

has developed an action plan for dietary guidelines based on existing evidence from health promotion programmes. The plan describes population goals in terms of nutrients and lifestyle for the prevention of chronic diseases in Europe (Busse *et al.* 2010). Therefore, in recent decades, dietary guidelines and healthy food consumption have been promoted to reduce the risk and manage chronic diseases. The combination of consumer requirements, food technology advances, and the improvement in evidence-based science concerning diet and disease prevention has created an important opportunity to address public health issues through diet and lifestyle. Widespread interest in foods that might promote health has lead to use of the term "functional foods." Many researchers have provided evidence of the clear relationships between dietary components and health benefits (Clydesdale 2005). In the case of traditional wild vegetables, despite current lifestyles and eating habits which make their use difficult, there is growing scientific interest in the potential benefits of these vegetables for their nutritional properties and the wealth of bioactive compounds, such as antioxidants, which have proven health-promoting properties (Burton & Traber 1990; Carpenter *et al.* 2009; Pardo de Santayana *et al.* 2010).

Wild plant gathering has been a habitual practice from ancient times in Europe, especially in times of shortage, playing an important role in complementing and balancing a diet based on agricultural foods. Many species now considered as weeds were considered food in past times. Nowadays, in our society many of these species have been forgotten, even when they have an important nutritive value, though many plant species are still used in other countries. However, some agricultural populations include significant quantities of forage plants in their diets and they are much appreciated, often being sold in local markets (Ertug 2004). Moreover, edible wild plants are considered essential elements of many European cultures and are a predominant feature of the landscape created by humans over the centuries (Heinrich *et al.* 2006a,b).

Many European ethnobotanical studies have reported on traditional knowledge about the plants used, most of them focused in the Mediterranean countries (Hadjichambis *et al.* 2008; Leonti *et al.* 2006; Sánchez-Mata & Tardío, 2016; Tardío *et al.* 2006).

These species can be very valuable during seasons when fresh agricultural products are scarce, such as winter and spring in the case of wild vegetables.

In recent years, many authors have undertaken the characterization of different chemical compounds in order to assess the nutritional potential of these wild species that have been part of the traditional diet of our ancestors and are still present in our current diet (Tables 7.7 and 7.8) (Barros *et al*. 2009, 2010a,b,c, 2011a,b; Conforti *et al*. 2008, 2011; Dias *et al*. 2013, 2014; García Herrera *et al*. 2014a,b; Guil *et al*. 1996a,b, 1997a,b,c, 1998, 2003; Hinneburg *et al*. 2006; Martins *et al*. 2011; Morales *et al*. 2012a,b, 2014, 2015; Sánchez-Mata *et al*. 2012; Pereria *et al*. 2011, 2013; Salvatore *et al*. 2005; Tardío *et al*. 2011; Trichopoulou *et al*. 2000; Vasilopoulo *et al*. 2011; Vardavas *et al*. 2006a,b, among many others). Also, it is important to keep in mind the great variability of these wild species, and in many cases indigenous varieties differ from species harvested and consumed in other countries.

Table 7.7 Vegetables traditionally consumed in Europe, standing out as sources of vitamins or minerals. Data are given per 100 g of fresh weight.

Species	Edible part	Vitamins	Minerals	References
Allium ampeloprasum L.	Bulbs and pseudostem	Vitamin B_9 (100–190 μg/100 g)	K (145–600 mg/100 g); Cu (0.04–0.18 mg/100 g)	García Herrera *et al*. 2014a Morales *et al*. 2015
Anchusa azurea Mill.	Leaves	Vitamin B_9 (256–299 μg/100 g); C (5.41–18.11 mg/100 g)	Ca (126–219 mg/100 g); K (268–1172 mg/100 g); Mn (0.15–0.699 mg/100 g)	Ayan *et al*. 2006; Morales *et al*. 2012; García Herrera *et al*. 2014b; Morales *et al*. 2015
Apium	Young	Vitamin B_9	Ca (152	Morales

nodiflorum (L.) Lag.	leaves and stems	(125 µg/100 g); C (10.3–30.2 mg/100 g); E* (2.62 mg/100 g);	mg/100 g); Cu (0.08 mg/100 g)	2012; Morales *et al*. 2012; García Herrera *et al*. 2014; Morales *et al*. 2015
Asparagus acutifolius L.	Young shoots	Vitamin B_9 (217 µg/100 g); C (37.8 mg/100 g); E (8.21 mg/100 g)	K (492–1370 mg/100 g); Mg (17–109 mg/100 g); Mn (0.410 mg/100 g)	Bianco *et al*. 1996; Martins *et al*. 2011; Romojaro *et al*. 2013; Gárcia Herrera 2014; Morales 2012; Morales *et al*. 2015
Beta maritima L.	Leaves	Vitamin B_9 (309 µg/100 g); C (18.3–66 mg/100 g)	K (540–2356 mg/100 g); Fe (1.42–4.31 mg/100 g); Mg (13.2–135 mg/100 g); Mn (0.640–1.260 mg/100 g)	Guil *et al*. 1997a,b; Sánchez-Mata *et al*. 2012; García Herrera 2014; Morales *et al*. 2014; Morales *et al*. 2015
Borago officinalis L.	Leaves	RAE** (238 µg/100 g)	K (567 mg/100 g); Ca (344 mg/100 g)	Bianco *et al*. 1996; Salvatore *et al*. 2005

Bryonia dioica Jacq.	Young shoots	Vitamin B_9 (43.2 µg/100 g); C (21.4 mg/100 g); K (95 µg/100 g); E (2.64 mg/100 g)	K (487 mg/100 g); Cu (0.220 mg/100 g); Mn (0.250 mg/100 g)	Vardavas *et al.* 2006a,b; Martins *et al.* 2011; Morales *et al.* 2012; 2015; Sanchez-Mata *et al.* 2012; Gárcia Herrera 2014; García Herrera *et al.* 2014
Capsella bursa-pastoris (L.) Medik.	Leaves	Vitamin C (91–169 mg/100 g)	K (315–564 mg/100 g); Ca (115–203 mg/100 g); Fe (3.50–6.14 mg/100 g); Mn (0.670–1.110 mg/100 g)	Gui-Guerrero *et al.* 1999b; Ayan *et al.* 2006; Kiliç and Cpskun 2007
Chenopodium album L.	Leaves	Vitamin C (137–171 mg/100 g)	K (855–1444 mg/100 g); Ca (236–438 mg/100 g); Mg (112–393 mg/100 g); Fe (4.79–5.80	Aliotta and Pollio 1981; Guil-Guerrero *et al.* 1997a; Guil-Guerrero and Torija-isasa 1997; Bianco *et*

			mg/100 g); Cu (0.040–0.330 mg/100 g); Mn (0.550–1.590 mg/100 g)	*al*. 1998; Yildrim *et al*. 2001
Chondrilla juncea L.	Leaves	Vitamin B_9 (90.2 μg/100 g)	K (433–1277 mg/100 g); Mn (970 μg/100 g); Ca (22–472 mg/100 g); Mg (2.70–100 mg/100 g); Cu (0.430 mg/100 g); Zn (1.630 mg/100 g)	Morales *et al*. 2012b; 2015; Ranfa *et al*. 2014; García Herrera *et al*. 2014b
Cichorium intybus L.	Leaves	Vitamin B_9 (253 μg/100 g); C (11.5–23 mg/100 g); K (173 mg/100 g); RAE (245 μg/100 g)	K (50–1085 mg/100 g); Ca (45.5–276 mg/100 g)	Bianco *et al*. 1998; Vardavas *et al*. 2006b; Sánchez-Mata *et al*. 2012; García Herrera *et al*. 2014b; Morales *et al*. 2014; 2015
Crithmum maritimum L.	Leaves	Vitamin C (39–76.6 mg/100 g)	Mg (57.4–97 mg/100 g); Mn	Franke 1982; Guil-Guerrero *et*

			(432–1080 mg/100 g); Ca (85–414 mg/100 g); Fe (1.09–3.70 mg/100 g)	*al*. 1996a; 1997a; 1998b; Romojaro *et al*. 2013
Daucus carota L.	Leaves	Vitamin C (127 mg/100 g); K (328 µg/100 g)	–	Vardavas *et al*. 2006a, b
Eruca vesicaria (L.) Cav.	Leaves	Vitamin C (125 mg/00 g); E (3.08 mg/100 g); K (31 µg/100 g)	Ca (160–327 mg/100 g); Mg (22–48.1 mg/100 g); Fe (1.04–2.91 mg/100 g)	Bianco *et al*. 1998; Vardavas *et al*. 2006b; Villatoro *et al*. 2012
Foeniculum vulgare Mill.	Leaves	Vitamin B_9 (271 µg/100 g); C (18–101 mg/100 g)	K (674 mg/100 g dw); Ca (1272 mg/100 g dw); Mg (235.9 mg/100 g dw); Fe (0.07–10.2 mg/100 g dw); Zn (0.33 mg/100 g dw)	Trichopoulou *et al*. 2000; Zeguichi *et al*. 2003; Vardavas *et al*. 2006b; Conforti *et al*. 2009; Morales *et al*. 2012a; 2015; Sánchez-Mata *et al*. 2012; Romojaro *et al*. 2013; García

				Herrera *et al.* 2014
Fragaria vesca L.	Roots and vegetative parts	Vitamin B_9 (115 µg/100 g); E (3.3 mg/100 g)	K (237–2774 mg/100 g); Ca (196–822 mg/100 g); Mg (30.4–331 mg/100 g); Fe (45.3 mg/100 g); Zn (0.799 mg/100 g)	Dias et al. 2015
Humulus lupulus L.	Young shoots	Vitamin B_9 (144 µg/100 g); C (28.6–61.1 mg/100 g)	K (314–675 mg/100 g); Mg (32.5 mg/100 g)	Morales 2012; Morales *et al.* 2012a; 2015; Sánchez-Mata *et al.* 2012; García Herrera *et al.* 2013; García Herrera 2014
Malva sylvestris L.	Leaves	Vitamin C (72–178 mg/100 g); E (20.58 mg/100 g)	K (547–836 mg/100 g); Ca (122–361 mg/100 g); Mg (209–368 mg/100 g); Fe (0.76–6.29	Franke & Hensbook 1981; Guil *et al.* 1997a,b; 1999a; Hiçsomenez *et al.* 2009; Barros *et al.*

			mg/100 g); Cu (0.10–0.330 mg/100 g); Mn (0.203–0.760 mg/100 g); Zn (0.038–2.665 mg/100 g)	2010a; Romojaro *et al*. 2013
Montia fontana L.	Young leaves and stems	Vitamin B_9 (41.8 µg/100 g); C (28.9–39.7 mg/100 g); E (4.62 mg/100 g)	K (356 mg/100 g); Mn (1070 mg/100 g)	Pereira *et al*. 2011; Morales 2012; Tardío *et al*. 2011; Morales *et al*. 2012a,b; 2015
Papaver rhoeas L.	Young leaves and stems	Vitamin B_9 (152 µg/100 g); C (18.7–47.6 mg/100 g); K (145 µg/100 g)	K (188–1672 mg/100 g); Cu (0.130–1.070 mg/100 g); Mn (0.390–1.060 mg/100 g)	Bianco *et al*. 1998; Trichopulou *et al*. 2000; Vardavas *et al*. 2006a; Sánchez-Mata *et al*. 2012; García Herrera 2014; Morales *et al*. 2014; 2015
Plantago lanceolata L.	Leaves	Vitamin C (13.6 mg/100 g)	K (263–415 mg/100 g); Ca (57–	Guil-Guerrero *et al*. 2001;

			660 mg/100 g); Mg (20.7–88 mg/100 g); Fe (1.11–5.12 mg/100 g); Cu (0.090–0.190 mg/100 g); Mn (0.310–1.012 mg/100 g)	Queralt *et al.* 2005; Ayan *et al.* 2006
Plantago major L.	Leaves	Vitamin C (24.3–92 mg/100 g)	K (283–357 mg/100 g); Mg (81–109 mg/100 g); Fe (1.20–2.80 mg/100 g); Cu (0.100–0.230 mg/100 g); Mn (0.300–0.520 mg/100 g)	Franke & Hensbook 1981; Aliotta and Pollio 1981; Guil-Guerrero *et al.* 1998b; Guil-Guerrero *et al.* 2001; Stef *et al.* 2010
Portulaca oleracea L.	Leaves	Vitamin C (29–109 mg/100 g)	K (280–611 mg/100 g); Mg (56–276 mg/100 g); Fe (2.90–5.68 mg/100 g); Cu (0.360–0.420 mg/100 g); Mn (0.540–	Bruno *et al.* 1980; Franke & Hensbook 1981; Guil-Guerrero *et al.* 1997a; Bianco *et al.* 1998; Dias *et al.* 2009

			0.640 mg/100 g)	
Prasium majus L.	Leaves	Vitamin K (373 µg/100 g)	–	Vardavas *et al*. 2006a
Rumex obtusifolius L.	Leaves	Vitamin C (32 mg/100 g); K (328 µg/100 g)	–	Vardavas *et al*. 2006a
Rumex papillaris Boiss. & Reut.	Leaves	Vitamin B_9 (187 µg/100 g); C (18.9–32.3 mg/100 g)	K (351 mg/100 g); Mg (45 mg/100 g); Mn (0.750 mg/100 g)	Morales *et al*. 2012b; 2014; 2015; Sánchez-Mata *et al*. 2012; García Herrera 2014
Rumex pulcher L.	Leaves	Vitamin B_9 (478 µg/100 g); C (28.7–46.5 mg/100 g)	K (891 mg/100 g)	Morales *et al*. 2014; 2015; Sánchez-Mata *et al*. 2012; García Herrera 2014
Scolymus hispanicus L.	Peeled basal leaves	Vitamin B_9 (103 µg/100 g); K (38 µg/100 g); RAE (8.08 µg/100 g)	K (1040 mg/100 g); Ca (235 mg/100 g); Fe (2.36 mg/100 g)	Vardavas *et al*. 2006; Morales *et al*. 2012; 2014; 2015; Sánchez-Mata *et al*. 2012; García Herrera *et*

				al. 2014b
Silene vulgaris (Moench.) Garcke	Young leaves and stems	Vitamin B_9 (267 µg/100 g); C (25.5 mg/100 g); K (172 µg/100 g); RAE (85.7 µg/100 g)	K (601 mg/100 g); Ca (70.7–254 mg/100 g); Mg (24.2–109 mg/100 g); Mn (0.540–1.010 mg/100 g)	Zeguichi *et al*. 2003; Alarcón *et al*. 2006; Ayan *et al*. 2006; Vardavas *et al*. 2006; Morales 2012; Morales *et al*. 2012; 2015; Egea-Gilabert *et al*. 2013; García Herrera 2014
Silybum marianum (L.) Gaertner	Peeled basal leaves	Vitamin B_9 (41.7 µg/100 g)	K (718 mg/100 g)	Bianco *et al*. 1998; Morales *et al*. 2012; 2015; Sánchez-Mata *et al*. 2012; García Herrera *et al*. 2014
Smilax aspera L.	Leaves	Vitamin E (29.1 mg/100 g)	Mg (61.2 mg/100 g); Fe (1.01–2.31 mg/100 g)	Demo *et al*. 1998; Cabiddu & Decandia 2000; Poschenrieder *et al*. 2012

Sonchus asper (L.) Hill	Basal leaves and stems	Vitamin C (62.8 mg/100 g)	K (511 mg/100 g); Fe (2.98 mg/100 g); Mn (880 µg/100 g)	Bianco *et al*. 1998; Guerrero-Guil *et al*. 1998a
Sonchus oleraceus L.	Leaves	Vitamin B_9 (85.9 µg/100 g); C (10.1–86 mg/100 g); K (175 µg/100 g); RAE (87.5 µg/100 g)	K (481 mg/100 g); Ca (32–280 mg/100 g); Fe (0.57–5.62 mg/100 g); Mn (0.370–1.269 mg/100 g)	Saleh *et al*. 1977; Bruno *et al*. 1980; Guil-Guerrero *et al*. 1996b; 1997a; Bianco *et al*. 1998; Zeghichi *et al*. 2003; Ayan *et al*. 2006; Vardavas *et al*. 2006b; García Herrera *et al*. 2014b; Morales *et al*. 2014; 2015
Tamus communis L.	Young shoots	Vitamin B_9 (381 µg/100 g); C (58.6–79.4 mg/100 g)	K (371 mg/100 g); Ca (47 mg/100 g); Mg (22.4 mg/100 g); Cu (0.130 mg/100 g); Mn (0.165 mg/100 g)	Barros *et al*. 2011b; Martins *et al*. 2011, Morales 2012; Morales *et al*. 2012a, b; Sánchez-Mata *et al*.

				2012; Pereira *et al*. 2013; García Herrera *et al*. 2013; García Herrera 2014
Taraxacum officinale Weber	Leaves	Vitamin C (8.00–62.2 mg/100 g)	Fe (0.34–14.4 mg/100 g); Mn (0.699 mg/100 g)	Aliotta & Pollio 1981; Bockholt & Schnittke 1996; Bianco *et al*. 1998; Ayan *et al*. 2006; Gjorgieva *et al*. 2010; Gatto *et al*. 2011; Dias *et al*. 2014
Taraxacum obovatum (Willd.) DC.	Leaves	Vitamin B_9 (110 μg/100 g); C (11.5–20.8 mg/100 g)	K (566 mg/100 g); Ca (177 mg/100 g); Fe (3.57 mg/100 g); Cu (0.150 mg/100 g)	García Herrera *et al*. 2014b; Morales *et al*. 2014
Urtica dioica L.	Leaves	Vitamin B_2 (230 μg/100 g); B_3 (620 μg/100 g); C (238–333	K (13.3–740 mg/100 g); Ca (246–982 mg/100 g); Mg (14–	Kudritsata & Zagorodskaya 1987; Wetherilt 1992;

		mg/100 g); RAE (476 μg/100 g)	482 mg/100 g); Cu (0.100–0.502 mg/100 g)	Bianco *et al*. 1998; Guil-Guerrero *et al*. 2003

* Vitamin E: calculated as content of α-tocopherol + (content of β-tocopherol/2) + (content of γ-tocopherol/10) + (content of δ-tocopherol/30).

** RAE (= retinol activity equivalents) are usually calculated as (content of β-carotene/12) + (contents of other provitamin A carotenoids /24); according to Mahan *et al*. (2012).

Table 7.8 Vegetables traditionally consumed in Europe, standing out as sources of bioactive compounds. Data are given per 100 g of fresh weight.

Species	**Edible part**	**Fiber (g/100 g)**	**Carotenoids/ tocopherols/ PUFA/ organic acids/ phenolics/ others**	**References**
Allium ampeloprasum L.	Bulbs and pseudostem	3.72–4.74	Total phenolic compounds (42.2 mg GAE/g extract), total flavonoids (6.30 mg CE/g extract)	García Herrera *et al*. 2014a; Morales *et al*. 2015
Achillea millefolium L.	Vegetative part	–	γ-tocopherol (13.04 mg/100 g	Dias *et al*. 2013

			dw); C18:2n6 (47.16%); total phenolic acids (103.80 mg/g extract); total flavonoids (24.56 mg/g extract); total phenolic compounds (128.36 mg/g extract)	
Anchusa azurea Mill.	Leaves	3.50–4.40	PUFA and *n*-3 proportion (80.4 and 67.9%); oxalic acid (110–640 mg/100 g); total phenolic compounds (148 mg GAE/g extract; 178 mg chlorogenic acid/g); total flavonoids (84.8 mg	Ayan *et al.* 2006; Conforti *et al.* 2011; García Herrera *et al.* 2014b; Morales *et al.* 2014

			CE/g extract)	
Apium nodiflorum (L.) Lag.	Young leaves and stems	–	α-tocopherol (2.59 mg/100 g); oxalic acid (189–879 mg/100 g); total phenolics (80.5 mg GAE/g extract); total flavonoids (45.5 mg CE/g extract)	Morales 2012; Morales *et al*. 2012a
Asparagus acutifolius L.	Young shoots	4.83	α-tocopherol (7.88 mg/100 g); citric acid content (308 mg/100 g); total phenolics (17.6 mg GAE/g extract; 265 mg/g); phenolic acids (6.09 mg caffeic acid/g);	Bianco *et al*. 1996; Barros *et al*., 2011b; Martins *et al*. 2011; Morales 2012; Romojaro *et al*. 2013; Gárcia Herrera 2014; Morales *et al*. 2014

			total flavonoids (6.09 mg CE/g extract; 30 mg/g; 226 mg QE/g)	
Beta maritima L.	Leaves	3.29–9.50	Oxalic acid (50–1070 mg/100 g); total phenolics (61.9 mg GAE/g extract); total flavonoids (21.5 mg CE/g extract)	Guil *et al*. 1997a; Sánchez-Mata *et al*. 2012; García Herrera 2014; Morales *et al*. 2014
Borago officinalis L.	Leaves	–	β-carotene (2.86 mg/100 g); lutein (3.81 mg/100 g); neoxanthin (1.13 mg/100 g); violaxanthin (1.15 mg/100 g); α-tocopherol (1.08–1.22 mg/100 g); oxalic acid (561–569	Salvatore *et al*. 2005; Gatto *et al*. 2011; Pereira *et al*. 2011; 2013

			mg/100 g); total phenolics (39.6 mg/g); phenolic acids (6.40 mg caffeic acid/g); total flavonoids (18.9 mg QE/g)	
Bryonia dioica Jacq.	Young shoots	4.60	β-carotene (0.15–1.95 mg/100 g); lutein (0.68–3.70 mg/100 g); neoxanthin (0.17–3.70 mg/100 g); violaxanthin (0.03–2.15 mg/100 g); α-tocopherol (0.69–6.39 mg/100 g); γ-tocopherol (0.41 3.18 mg/100 g); C18:3n3 (25.4–70.3%); oxalic acid (70–630	Vardavas *et al*. 2006a, b; Barros *et al*. 2011b; Martins *et al*. 2011; Morales *et al*. 2012a; Sanchez-Mata *et al*. 2012

			mg/100 g); total phenolics (35.10 mg GAE/g extract); flavonoids (16.31 mg CE/g extract; 241 mg/g)	
Capsella bursa-pastoris (L.) Medik.	Leaves	–	Nitrate (256 mg/100 g)	Bianco *et al*. 1998
Chenopodium album L.	Leaves	6.38	Oxalic acid content (361–2027 mg/100 g)	Guil-Guerrero *et al*. 1997a; Guil-Guerrero and Torija-isasa 1997; Bianco *et al*. 1998
Chondrilla juncea L.	Leaves	4.10–13.4	C18:3n3 (56.3%); total phenolics (37.66 mg GAE/g); total flavonoids (7.43 mg CE/g extract)	Morales *et al*. 2012b; 2014; García Herrera *et al*. 2014b
Cichorium intybus L.	Leaves	–	Lutein (3.05–4.54	Salvatore *et al*. 2005;

	mg/100 g); neoxanthin (1.44 mg/100 g); violaxanthin (1.70 mg/100 g); α-tocopherol (0.88–1.10 mg/100 g); γ-tocopherol (0.58–1.97 mg/100 g); nitrate (12.9 mg/100 g); total phenolics (73.68 mg GAE/g extract; 122 mg/g; 306 mg cholorogenic acid/g); phenolic acids (15.8 mg caffeic acid/g); flavonoids (20.8 mg chlorogenic acid/g; 31.3 mg CE/g; 106 mg QE/	Conforti *et al*. 2009; 2011; Vardavas *et al*. 2006b; Morales *et al*. 2014

			g)	
Crithmum maritimum L.	Leaves	4.68	Carotenoids (3.30–5.60 mg β-carotene/100 g); C18:1n9 (16.11%); total phenolics (1260 mg/g); flavonoids (35.6 mg QE/g); tanins (193 mg/g); nitrate (63 mg/100 g)	Guil-Guerrero *et al.* 1996ab; 1997a; Males *et al.* 2003
Daucus carota L.	Leaves	–	β-carotene (1.77 mg/100 g); lutein (3.50 mg/100 g); α-tocopherol (0.53 mg/100 g); C18:2n6 (60.9 %)	Vardavas *et al.* 2006a, b
Eruca vesicaria (L.) Cav.	Leaves	–	β-carotene (1.15–1.40 mg/100 g); lutein (2.48 mg/100 g); α-tocopherol (3.07); total	Bianco *et al.* 1998; Vardavas *et al.* 2006b

			phenolics (211 mg GA/g)	
Fragaria vesca L.	Roots and vegetative parts	–	SFA (C16:0, 16.00%); C18: 3n3 (24.8%)	Dias et al. 2015
Foeniculum vulgare Mill.	Leaves	2.70–6.20	β-carotene (1.19 mg/100 g); lutein (3.66 mg/100 g); α-tocopherol (1.66 mg/100 g); total phenolics (42.16 mg GA/g extract; 195 mg chlorogenic acid/g); hydroxycinnamic acids (495 mg chlorogenic acid/g); flavonoids (9.72 mg CE/g extract; 27.5 mg chlorogenic acid/g; 82.5	Trichopoulou *et al*. 2000; Zeguichi *et al*. 2003; Vardavas *et al*. 2006b; conforti *et al*. 2009; 2011; Morales *et al*. 2012a; Sánchez-Mata *et al*. 2012; García Herrera *et al*. 2014; Vanzani *et al*. 2011

			mg/g); nitrate (24 mg/100 g)	
Humulus lupulus L.	Young shoots	4.85	β-carotene (0.49 mg/100 g); lutein (0.76 mg/100 g); neoxanthin (0.73 mg/100 g); violaxanthin (0.31 mg/100 g); α-tocopherol (4.51 mg/100 g); γ-tocopherol (8.98 mg/100 g; malic acid (295–1040 mg/100 g); total phenolics (55.83 mg GAE/g extract); flavonoids (9.56 mg CE/g extract)	Morales 2012; Morales *et al*. 2012a; García Herrera *et al*. 2013
Malva sylvestris L.	Leaves	4.76	α-tocopherol (20.1	Franke & Hensbook 1981; Guil

			mg/100 g); γ -tocopherol (4.80 mg/100 g); total phenolics (1692 mg GA/100 g); flavonoids (925 mg CE/100 g); nitrate (87.6 mg/100 g)	*et al*. 1997a, b; 1999a; Barros *et al*. 2010a; Romojaro *et al*. 2013 Hiçsomenez *et al*. 2009
Montia fontana L.	Young leaves and stems	4.44	α-tocopherol (2.90–6.08 mg/100 g); C18:3n3 (47.4–56.4%); total phenolics (75.53 mg GAE/g extract); flavonoids (16.67 mg CE/g extract)	Pereira *et al*., 2011; Tardio *et al*. 2011; Morales *et al*. 2012a, b
Papaver rhoeas L.	Leaves	2.50–11.10	Oxalic acid (124–850 mg/100 g); total phenolic (25.86 mg	Trichopulou *et al*. 2000; Zeghichi *et al*. 2003; Conforti *et al*. 2011;

			GAE/g extract; 239 mg chlorogenic acid/g); flavonoids (12 mg CE/g extract; 31 mg/g; 143 mg chlorogenic acid/g); nitrates (219–307 mg/100 g)	Vardavas *et al*. 2006a; Sánchez-Mata *et al*. 2012; García Herrera 2014; Morales *et al*. 2014
Plantago lanceolata L.	Leaves	3.71	Phenolic acids (631 mg/g; 327 mg GA/g); hydroxycinnamic acids (509 mg chlorogenic acid/g); flavonoids (49.6 mg CE/g)	Guil-Guerrero *et al*. 2001; Galvez *et al*., 2005; Gatto *et al*. 2011; Vanzani *et al*., 2011
Plantago major L.	Leaves	3.19–4.57	Nitrates (94–108 mg/100 g)	Guil-Guerrero *et al*. 1997a; 2001
Portulaca oleracea L.	Leaves	–	Lutein (5.40 mg/100 g); zeaxanthin (0.2 mg/100 g);	Guil-Guerrero *et al*. 1997a; Bianco *et al*. 1998; Dias *et al*.

			oxalic acid (681 mg/100 g)	2009
Prasium majus L.	Leaves	–	Lutein (4.13 mg/100 g); β-carotene (2.17 mg/100 g)	Vardavas *et al*. 2006a
Rumex obtusifolius L.	Leaves	–	Lutein (3.44 mg/100 g); β-carotene (1.44 mg/100 g); α-tocopherol (0.85 mg/100 g)	Vardavas *et al*. 2006a
Rumex papillaris Boiss. & Reut.	Leaves	4.40	C18:3n3 (51.8%); oxalic acid (3 –560 mg/100 g); total phenolics (104 mg GAE/g extract); flavonoids (39.5 mg CE/g)	Morales *et al*. 2012b; 2014; Sánchez-Mata *et al*. 2012; García Herrera 2014
Rumex pulcher L.	Basal leaves	5.45	Oxalic acid (57.7–730 mg/100 g); total phenolics	Morales *et al*. 2014; 2015; Sánchez-Mata *et al*.

			(73.4 mg GAE/g extract); flavonoids (26.1 mg CE/g extract)	2012; García Herrera 2014
Scolymus hispanicus L.	Peeled basal leaves	7.00	β-carotene (0.1 mg/100 g); lutein (0.33 mg/100 g); total phenolics (21.51 mg GAE/g extract); flavonoids (8.38 mg CE/g extract)	Vardavas *et al*. 2006; Morales *et al*. 2014; García Herrera *et al*. 2014b
Silene vulgaris (Moench.) Garcke	Leaves	2.60–6.63	β-carotene (1.029 mg/100 g); lutein (2.012 mg/100 g); α-tocopherol (0.35–1.65 mg/100 g); total phenolics (26.72 mg GAE/g extract; 68.7 mg	Zeguichi *et al*. 2003; Alarcón *et al*. 2006; Vardavas *et al*. 2006; Vanzani *et al*. 2011; Conforti *et al*. 2011; Morales *et al*. 2012; Egea-Gilabert *et al*. 2013; García

			chlorogenic acid/g; 88.6 mg /g); hydroxycinnamic acids (230 mg chlorogenic acid/g); flavonoids (21.6 mg CE/g extract); nitrate (178 mg/100 g)	Herrera 2014
Silybum marianum (L.) Gaertner	Peeled basal leaves	–	Oxalic acid (171–1889 mg/100 g); total phenolics (3.72 mg GAE/g extract); flavonoids (1.13 mg CE/g extract); nitrate (113 mg/100 g)	Bianco *et al*., 1998; Morales *et al*., 2012; Sánchez-Mata*et al*. 2012
Smilax aspera L.	Basal leaves and stems	18.8	α-tocopherol (29.1 mg/100 g); β-tocopherol (4.5 mg/100 g)	Demo *et al*. 1998; Cabiddu & Decandia 2000
Sonchus	Leaves	–	Total	Bianco *et*

asper (L.) Hill			phenolics (102 mg chlorogenic acid/g; 308 mg/g); nitrate (72.6 mg/100 g)	*al*. 1998; Gatto *et al*. 2011; Conforti *et al*. 2011
Sonchus oleraceus L.	Young shoots	2.60–5.57	β-carotene (1.05 mg/100 g); lutein (1.83 mg/100 g); α-tocopherol (0.29–1.75 mg/100 g); oxalic acid (98–840 mg/100 g); total phenolics (51.33 mg GAE/ extract g; 217 mg chlorogenic acid/g; 220 mg/g); flavonoids (14.83 mg CE/g extract; 34.3 mg/g; 90.6 mg chlorogenic acid/g);	Guil-Guerrero *et al*. 1998a; Trichopoulou *et al*. 2000; Zeghichi *et al*. 2003; Vardavas *et al*. 2006a,b; Conforti *et al*. 2009; Gatto *et al*. 2011; Vanzani *et al*. 2011; García Herrera *et al*. 2014b; Morales *et al*. 2014

			nitrate (51.1–191 mg/100 g)	
Tamus communis L.	Leaves	3.50–9	β-carotene (0.44 mg/100 g); lutein (1.14 mg/100 g); neoxanthin (1.19 mg/100 g); violanxanthin (0;.62 mg/100 g); α-tocopherol (0.12–3 mg/100 g); γ-tocopherol (1.28–2 mg/100 g); C18:3n6 (42%); total phenolics (49.5 mg GAE/g extract; 220 mg/g); flavonoids (9.33 mg CE/g extract; 201 mg /g)	Barros *et al*. 2011b; Martins *et al*. 2011, Morales 2012; Morales *et al*. 2012a, b; Sánchez-Mata *et al*. 2012; Pereira *et al*. 2013; García Herrera *et al*. 2013; García Herrera 2014
Taraxacum officinale Weber/sect.	Leaves	–	Carotenoids (3.59 mg/100 g);	Aliotta & Pollio 1981; Bockholt &

Ruderalia			α-tocopherol (3.52 mg/100 g); C18:2n6 (62%); oxalic acid (986–1003 mg/100 g); nitrate (29.6 mg/100 g); %); total flavonoids (9.69 mg/g extract extract); total phenolic acids (43.24 mg extract/g extract); total phenolic compounds (52.93 mg/g extract)	Schnittke 1996; Bianco *et al.* 1998; Ayan *et al.* 2006; Gjorgieva *et al.* 2010; Gatto *et al.* 2011; Dias *et al.* 2014
T. obovatum (Willd.) DC.	Leaves	7.01	Total phenolics (58.23 mg GAE/g extract); flavonoids (30 mg CE/g extract)	García Herrera *et al.* 2014b; Morales *et al.* 2014
Urtica	Leaves	–	β-carotene	Kudritsata

dioica L.				(1.18–10.9 mg/100 g); lutein (5.25–5.97 mg/100 g); neoxanthin (0.43 mg/100 g); violaxanthin (1.92 mg/100 g); α-tocopherol (14.4 mg/100 g); nitrate (92 mg/100 g)	& Zagorodskaya 1987; Wetherilt 1992; Bianco *et al*. 1998; Guil-Guerrero *et al*. 2003

* CE, catechin equivalent; GAE, gallic acid equivalent; PUFA, polyunsaturated fatty acid; QE, quercitin equivalent; SFA, saturated fatty acid.

Table 7.7 presents data on levels of vitamins and minerals in wild leafy vegetables traditionally consumed in Europe obtained from scientific literature. Only data on species with significant nutrient contents have been recorded: 100 g of fresh material providing more than 15% of the generally accepted daily recommendations (FAO/WHO 2004; Trumbo *et al*. 2002).

In general, *Chondrilla juncea* L. stands out as a K, Fe, Cu, and Mn contributor; *Portulaca oleracea* for its Mg, Fe, Cu, and Mn content and also, although with higher variability, the leaves of some *Urtica* species (Mg and Fe levels). Wild edible plant foods are often good sources of calcium, as many wild leafy vegetables contain even higher levels than many foods widely accepted as good calcium sources, such as dairy products. *Urtica dioica*, *Foeniculum vulgare* Mill., *Chenopodium album*, *Borago oficinalis* L., *and Eruca vesicaria* L. Cav., among others, are considered as valuable Ca contributors, with values up to 300 mg/100 g fw, *Urtica dioica* being one of the richest Ca sources

(246–982 mg/100 g fw). Moreover, the potential bioavailability of this macroelement is high in wild vegetables such as *F. vulgare*, *Malva sylvestris* L., *Capsella bursa-pastoris* L., *Eruca vesicaria* and some *Plantago* species, that stand out due to their oxalic acid levels, being potentially better absorbed than calcium from other plant sources. Some wild greens can contain unusually high potassium levels, such as *Beta maritima* L., *Chondrilla juncea*, *Scolymus hispanicus* L., and *Chenopodium album*, reaching levels of 1 g/100 g fw or more (see Table 7.7). Regarding microelements, iron deficiency in Europe is not as serious as in other countries, such as in Africa or Asia. However, some plant foods may moderately contribute to daily iron intake. In this respect, *Urtica dioica*, *Capsella bursa-pastoris* Medik., *Chondrilla juncea*, *Portulaca oleracea*, and *Taraxacum obovatum* (Willd.) DC. edible parts may contain high Fe levels (see Table 7.7).

As previously mentioned, wild greens are a great source of vitamins, particularly vitamin C, B_9, and K. In the last few years, some authors have studied the vitamin C content (as total vitamin C, ascorbic acid, and dehydroascorbic acids) in different wild vegetables traditionally consumed in the Mediterranean area (Sánchez-Mata & Tardío 2016). It is noteworthy that many of them stand out for their high vitamin C levels, such as leaves of *Portulaca oleracea* (up to 109 mg/100 g fw), *Eruca sativa* Hill. (125 mg/100 g fw), *Daucus carota* L. (over 127 mg/100 g fw), *Capsella bursa-pastoris* L. (169 mg/100 g fw), and *Chenopodium album* (131–171 mg/100 g fw). Another neglected vegetable with high vitamin C values is *Urtica dioica* (238–333 mg/100 g fw), which easily provides the total daily recommendation of vitamin C (see Table 7.7).

Leafy vegetables, and particularly wild greens, could be a good source of dietary folates, and an increase in their consumption would be a good strategy to avoid the consequences of vitamin B_9 deficiency. Despite a decrease in the consumption of fruits and vegetables in Europe, there are no serious problems of folate deficiency, as its supplementation is recommended primarily in pregnant women (FAO/WHO 2004). The prevalence of neural tube defects varies across the EU, occurring in 0.4–2.0 cases per

1000 births; the range of variation is attributed to differences in reporting and collecting data in the different European countries (European Food Safety Authority 2008). To our knowledge, there are not many data available about folic acid and folate content in wild vegetables in general, and particularly in Europe. Considering published values, it could be stated that many of them have high folate values, particularly *Rumex pulcher* L. (478 µg/100 g fw), followed by *Beta maritima*, *Anchusa azurea* Mill., *F. vulgare*, *Silene vulgaris* (Moench) Garcke., *Cichorium intybus* L., and *Asparagus acutifolius* L. All these could be considered as a source of this nutrient, reaching almost 200 µg/100 g fw (Morales *et al*. 2015), thgus being able to provide the whole daily recommendation (200–400 µg/day) in a 100 g portion (Cuervo *et al*. 2009).

Regarding liposoluble vitamins, carotenoids are considered of great interest due to the provitamin A activity of some of them (α-carotene, β-carotene, and cryptoxanthin, mainly), but also for other bioactive properties such as antioxidant and antiinflammatory capacity (Elliott 2005; Stahl & Sies 2012). There are very few studies regarding carotenoid content in wild greens but some indicate interesting vitamin A values (expressed as estimated retinol activity equivalents (RAE)), with *Urtica dioica* followed by *Cichorium intybus* and *Daucus carota* the richest species (476, 245 and 238 µg/100 g, respectively) (see Table 7.7). However, in leafy vegetables, nonprovitamin A carotenoids, such as xanthophylls (lutein, neoxanthin, and violaxanthin), are usually the major carotenoids, even in many cases at higher levels than β-carotene (García Herrera 2014; Guil Guerrero *et al*. 2003;Salvatore *et al*. 2005). Lutein is present in wild green vegetables, with *Urtica dioica*, *Portulaca oleracea*, *Cichorium intybus*, *and Prasium majus* L. beingsome of the richest species with lutein values of 4.13–5.97 mg/100 g fw (see Table 7.7).

Wild European vegetables, such as *Smilax aspera* L., *Malva sylvestris* L., *Apium nodiflorum*, and *Montia fontana* L., and tender shoots, such as *A. acutifolius* and *Bryonia dioica*, have high vitamin E content (2.62 and 29.1 mg/100 g in *A. nodiflorum* and *S. aspera*, respectively), calculated according to the FAO/

WHO methods (2004); α-tocopherol was the main isoform (4.5 and 29.1 mg/100 g in *M. fontana* and *S. aspera*, respectively) (see Table 7.8). In some cases γ-tocopherol was also found in relatively important amounts in *Humulus lupulus*, *Malva sylvestris*, *S. aspera*, and *B. dioica* (8.98–3.18 mg/100 g).

Vitamin K, mainly as phylloquinone (K1) vitamer, is synthesized by green vegetables and is widely distributed throughout the diet. In general, relative values in green leafy vegetables are around 400–700 μg/100 g. Vitamin K deficiency is very uncommon in humans, aside from a small percentage of infants who suffer from hemorrhagic disease of the newborn, a potentially fatal disorder. In adult humans, a prolonged blood-clotting time is the predominant, if not sole, clinical sign of vitamin K deficiency (FAO/WHO 2004). There are very few studies regarding vitamin K levels in wild vegetables. However, Vardavas *et al*. (2006) demonstrated that most of them provide the necessary amount to cover 100% of daily recommended intake (50–65 μg/day; FAO/WHO 2004) and can be considered as very good sources of this nutrient (more than 11.3 μg/100 g, according to European Regulation 1169/2011), as can be seen in *Prasium majus*, *Rumex obtusifolius* L., *Daucus carota*, and *F. vulgare* which contain the highest levels (more than 300 μg/100 g fw).

Other important bioactive compounds in wild edible European greens are dietary fiber, phenolics, organic acids, and polyunsaturated fatty acids (PUFAs), which are shown in Table 7.8. Several studies show that the ingestion of suitable quantities of dietary fiber provides many beneficial effects such as the regulation of intestinal function, improvement of glucose tolerance in diabetics, and prevention of chronic diseases such as colon cancer (Mongeau 2003; Pérez Jiménez *et al*. 2008). From the nutritional standpoint, fiber is the most important component of wild plants. The plant species with the highest fiber contents include *Beta maritima*, *Tamus communis* L., *Scolymus hispanicus* L., and *Taraxacum obovatum* with values up to 7 g/100 g, with *Smilax aspera* and *Chondrilla juncea* the ones with the highest values (18.8 and 13.4 g/100 g, respectively). In most cases wild European edible greens (see Table 7.8) could be considered as source of dietary fiber (more than 3 g/100 g fw).

Phenolic compounds, as secondary plant metabolites, are gaining greater prominence as bioactive agents; total phenols and flavonoids have an important role in antioxidant defense mechanisms in the plant, and there are a great number of *in vitro* and *in vivo* studies, using animal and human cell cultures, suggesting that these bioactive compounds of wild plants show a positive effect on human health, such as antioxidant, antitumoral, antimutagenic, antimicrobial, antiinflammatory, and neuroprotective properties (Carocho & Ferrerira 2013). There are several studies on the phenolic composition of wild edible plants, identifying phenolic acids (in some cases different hydroxycinnamic acids) and flavonoids such as proanthocyanidins and anthocyanins. These compounds have been reported in wild species traditionally consumed in Europe, mainly focused in the Mediterranean area (Conforti *et al*. 2008, 2011; García Herrera *et al*. 2014a; Martins *et al*. 2011; Morales 2011; Morales *et al*. 2012a,b, 2014; Pereira *et al*. 2011; Salvatore *et al*. 2005; Vardavas *et al*. 2006; Zeghichi *et al*. 2003, among others). Polyphenols can be measured (e.g. spectrophotometry, HPLC-DAD, HPLC-MS) and expressed differently (e.g. as gallic acids, cathechin, chlorogenic acid, quercetin equivalents or as a sum of different individual compounds), therefore in some cases it is very difficult to compare results. European wild species such as *Eruca vesicaria* (L.) Cav., *Montia fontana*, and *Plantago lanceolata* L. stand out due to their total phenolic content (211, 277 and 327 mg gallic acid equivalent (GAE)/g methanolic extract, respectively), while *Apium nodiflorum*, *P. lanceolata*, *M. fontana*, and *Anchusa azurea* were reported to have high flavonoid contents (45.5, 49.6, 52.3 and 84.8 mg catechin equivalents (CE)/g, respectively). Regarding other polyphenol families, tannin content was reported in *Crithmum maritimum* L. (193 mg/100 g fw) and hydroxycinnamic acids in *P. lanceolata*, *F. vulgare*, and *S. vulgaris* (230, 495 and 509 mg chlorogenic acid/100 g fw, respectively).

Polyunsaturated fatty acids (PUFA) play an important role in human nutrition, being associated with several health benefits. Unsaturated fatty acids are associated with a reduced risk of developing cardiovascular disease, inflammatory and autoimmune

diseases such as asthma, Crohn's disease and arthritis, and certain cancers, including colon, breast, and prostate (Simopoulus 2004). Vegetables are rich in PUFA of *n*-3, *n*-6, and *n*-9 series, such as α-linolenic, linoleic, and oleic acids, respectively. Wild edible plants contain in general a good balance of *n*-6 and *n*-3 fatty acids, particularly *Anchusa azurea*, *Bryonia dioica*, *Chondrilla juncea*, *Montia fontana*, *Rumex papillaris*, and *Tamus communis* due to their high *n*-3 fatty acid proportion (see Table 7.8), mainly as α-linoleic acid (ALA; C18:3n3).

In Europe, wild species are traditionally gathered due to their great versatility in handling and consumption, and may have great potential as a source of functional compounds. This justifies the need to preserve their traditional uses, as an alternative to the variety of currently available cultivated vegetables or as ingredients of new dietary supplements and/or functional foods.

7.3 Implications of Wild Greens Consumption for Human Health: Safely Gathering Wild Edible Plants

Despite all the information about the nutritional benefits of wild edible plants, when studying the health implications of their consumption, the possibility of negative effects should also be considered. From ancient times, empirical knowledge about wild plant properties has governed their utilization all over the world. Observation of what animals or other humans ate suggested which plants were edible and which may be hazardous, and this information was transmitted through generations. The presence of these wild vegetables in the human diet provided fiber, vitamins, minerals, and other bioactive compounds necessary to avoid many deficiency diseases and allowed hunter-gatherers to achieve generally a good health status; in fact, some of the features of Paleolithic nutrition have evolved negatively in modern diets (Simopoulos 2004).

Many wild plants were also used as natural remedies to cure diseases, sometimes with success, other times failing; in some

cases the cure was achieved spontaneously and the medicinal property was erroneously attributed to the plant used. Other wild plants were also known as natural poisons, and so warnings were transmitted about their erroneous use. In other cases, they may be intentionally used: impregnated in arrows and darts for hunting or fighting (e.g. curare's paralyzing principles or *Strychnos* spp. extracts); for Greek or Roman executions (e.g. Socrates, condemned by the senate of Athens to drink an extract made of *Conium maculatum* L., the poison hemlock, in 399 BC); or even for murder (e.g. using aconitum root) (Diggle 2003; Oghno 1998; Philippe & Angenot 2005). In this context, food, medicine, and poison are three concepts that may sometimes be very close in nature, as stated by Hippocrates in the fifth century BC ("let food be your medicine and medicine be your food") and Paracelsus in the sixteenth century AD ("dosis sola facit venenum" - the dose makes the poison).

Later, acquired empirical knowledge was subjected to investigation, first by alchemists or apothecaries and later by naturalists, botanists, doctors, and pharmacists. As laboratory techniques developed, the mechanisms of action and the structure-activity relationships of chemical compounds were studied. In some cases, a true biological activity was not found, but in other cases, the empirically claimed properties were confirmed. Thus many traditional plants continued to be used but under the control of health professionals and authorities, and in some cases this has lead to the industrial development of medicines made with different plant parts (e.g. *Plantago* seeds as laxatives) or isolated active principles, either native (e.g. digoxin from *Digitalis* species or cocaine from *Erythroxylum coca* Lam. leaves) or chemically modified (e.g. acetylsalicylic acid derived from salicin obtained from *Salix alba* L. bark or antiasthmatic substances semisynthesized from atropine extracted from *Atropa belladonna* L.) (Bahar *et al*. 2008; Evans 2009).

Unfortunately, with the intense development of agriculture in the 20th century, many of the traditional practices of gathering wild plants from nature, for either food or medicinal use, have almost disappeared, displaced by cultivated crops. Demographic movement from fields to cities has also meant that a great part of

the knowledge achieved by humans through centuries has been forgotten in just two or three generations. Nowadays, new trends of recovering these practices are arising, as a new philosophy of life based on returning to nature, in developed societies, or as a tool to fight against food shortages, in developing ones.

The revalorization of traditional wild plant use is positive and valuable; however, when the knowledge chain has been interrupted for several generations, caution should be exercised. Many people are keen to return to natural ways of life and try to imitate their ancestors by collecting plants and fungi, with the wrong idea that all "natural" products are harmless. Those who gathered wild plant foods from nature in the past had been familiar with these practices since their childhood, watching their parents or grandparents and learning about edibility of species and the proper way they should be handled. The experience of generations honed their knowledge, but some people today feel that they can do the same with very scanty information ("A friend told me…," "I saw it on the internet…,","This is similar to a picture I saw…"), and this may lead to mistakes, sometimes with bad consequences.

Apart from the presence of naturally occurring compounds, wild plants can carry contaminants, either chemical pollutants accumulated from the soil (when they grow near mines or highly industrialized areas) or parasites such as *Fasciola hepatica* (typically living on watercress) (Couplan 2002; Fawzi *et al.* 2003). The latter may be eliminated by washing the plant with diluted vinegar or by cooking, a practice often performed by the population that used to gather watercress but not always known by many "novel gatherers."

Regarding the naturally occurring compounds, in the latest reports (1989–2006) from different European and American official institutions on toxicology and toxicovigilance, about 2.5–5% of reported poisoning cases are due to plants, of which 80–90% were children accidentally ingesting toxic plants (specially infants under four years old swallowing fruits with attractive bright colors), and 10–20% were adults. Of adult poisonings, the main causes were intentional suicide, hallucinogen purposes, or errors in identification or utilization (Flesch 2005; Fourasté 2000; Moro *et al.* 2009). Deep knowledge of botany or long experience in plant

gathering is necessary to safely collect wild plants, since identification mistakes often occur between similar species (see some examples in Table 7.9), which can lead to serious poisoning. One cause of these misidentifications may be the fact that the presence of flowers often differentiates edible species from toxic ones, and since the optimal time for gathering leafy vegetables is when leaves are young, before flowering, confusion could easily occur (Bergerault 2010).

Table 7.9 Some examples of confusions between edible vegetables and toxic wild plants (Bergerault 2010).

Edible species (edible parts)	Resembling species (toxic principles)
Gentiana lutea L. (subterranean parts)	*Veratrum album* L. (alkaloids: protoveratrins, jervin)
Brassica napus L. (root)	*Aconitum napellus* L. (alkaloids: aconitin)
Symphytum officinale L. (leaves)	*Digitalis purpurea* L. (cardiotonic heterosides: digitoxin, gitoxin, digitalin)
Laurus nobilis L. (leaves)	*Nerium oleander* L. (cardiotonic heterosides) *Prunus laurocerasus* L. (cyanogen glucosides) *Daphne laureola* L. (diterpens)
Chenopodium bonus-henricus L. (leaves, inflorescences)	*Arum maculatum* L., *A. italicum* L. (cyanogenetic glucosides)
Leucanthemum vulgare Lam. (young leaves)	*Senecio jacobaea* L. (alkaloids: senecionin)
Sisymbrium officinale (L.) Scop. (leaves)	*Erysimum cheiranthoides* L. (cardiotonic heterosides)
Stellaria media (L.) Vill. (leaves, young stems)	*Anagallis arvensis* L. (hemolytic saponins: cyclamin, cucurbitacins)
Allium ursinum L. (leaves)	*Colchicum autumnale* L.

	(alkaloids: colchicin)
Abies alba Mill. (tender young shoots)	*Taxus baccata* L. (pseudoalkaloids: taxoids)

In other cases, toxicity is linked to eating the wrong part of the plant (e.g. stems of rhubarb are edible, while leaves are rich in oxalic acid and subterranean parts contain antraquinons with purgant effects). Toxic substances may also depend on the maturation stage of the plant (especially in some fruits, where often alkaloid levels are much higher in immature fruits than in mature ones, e.g. *Sambucus nigra* L. or *Solanum nigrum* L.). In some cases, toxins may be eliminated with the proper treatment; for example, oxalic acid in some *Rumex* species may be harmful at the levels detected in the raw product, but can be mostly removed by cooking; aconite alkaloids are extremely toxic, but notification of accidental intoxications with *Aconitum napellus* L. is scarce since they are heat labile (Bergerault 2010; Evans 2009; Morales 2012).

Some naturally occurring compounds in plant tissues showing certain degrees of toxicity are (Bruneton 2005; Cameán & Repetto 2006; Evans 2009).

- *Lectins*: in ricinus seeds (containing ricin, extremely toxic), *Sambucus* spp., and some Fabaceae; provoking hemagglutination, intense intestinal inflammation and epithelial destruction, sometimes lethal.
- *Diterpenes*: such as taxol, with anticancer activity, and responsible for serious poisoning after the ingestion of *Taxus* sp.
- *Saponins*:

    – Steroid saponins, in some Fabaceae and Dioscoreaceae tubers (provoking hemolysis), and cardiotonic heterosides in *Digitalis* spp. and *Convallaria majalis* L. (provoking bradycardia and heart failure).

    – Triterpene saponins, in fake-chestnut (*Aesculus hippocastanum* L.) and Cucurbitaceae fruits and roots, provoking gastrointestinal troubles of different degrees of importance.

- *Cyanogens*: in Lima beans (*Phaseolus lunatus* L.), some Rosaceae seeds and manihot tubers (*Manihot utilissima* Pohl); they can release HCN, provoking inhibition of cytochrome oxidase, and fatal respiratory failure.
- *Alkaloids*: usually giving plants a bitter taste, which usually is taken as a warning of toxicity for animals or humans. Some examples are:

  – Nonheterocyclic alkaloids, e.g. colchicin in *Colchicum* spp. bulbs, provoking serious organic alterations and death by cardiac failure.

  – Quinolizidine alkaloids, in some lupin (*Lupinus* sp.) seeds, with agonistic activity on nicotine receptors.

  – Piperidine alkaloids, e.g. in poison-hemlock *(Conium maculatum* L.), provoking nervous alterations and paralysis, usually lethal.

  – Terpenoid alkaloids, e.g. in *Aconitum napellus* L., considered one of the most powerful poisons in nature.

  – Pirrolizidine alkaloids, e.g. in senecio (*Senecio jacobaea* L.) and some Boraginaceae, provoking hepatotoxicity.

  – Indole alkaloids, e.g. strychnine from *Strychnos nux-vomica* L., provoking lethal neurological alterations.

  – Isoquinolein alkaloids, in opium extracted from some *Papaver* species, with narcotic effects.

Special attention should be given to the Solanaceae family, which has members known for containing tropan alkaloids (*Atropa belladonna* L., *Hyoscyamus niger* L., *Datura stramonium* L.) or glycoalkaloids (based on a steroid structure bonded to different sugars). Tropan alkaloids may cause serious intoxications, for example by smoking their leaves or accidentally confusing their fruits with other edible berries such as *Vaccinium myrtillus* L. (frequently in children) (Nogué *et al*. 2009). Solanine and chaconine are the major glycoalkaloids in potatoes (*Solanum tuberosum* L.), being highly toxic by inhibiting

acetylcholinesterase. Formal guidelines limit the total glycoalkaloid concentration in commercial potatoes to 200 mg/kg fw as a safe value (*Friedman et al*. 2003), taking into account that around 140 mg/kg a bitter taste is usually detected (Deshpande 2002). Other Solanaceae species have different alkaloids, such as tomatidine derivatives in tomatoes (*Solanum lycopersicum* L.) or solasodine derivatives found in eggplants, at low levels in cultivated species such as *Solanum melongena* L., but in higher levels in some of their wild relatives such as *Solanum macrocarpon* L. or *Solanum aethiopicum* L. (Sánchez-Mata *et al*. 2010). The fruits and leaves of these species are widely eaten in Africa.

However, the border between what is edible or not is not always so clear. For example, *S. nigrum* is reported to be eaten in Africa (Odhav *et al*. 2007; Steyn *et al*. 2001), while this species has been reported to be toxic due to high levels of glycoalkaloids (FAO 1988; Mohy-ud-dint *et al*. 2010). Taxonomic questions may be involved in this discrepancy: the FAO (1988) states that frequently the edible *S. americanum* Mill. is erroneously identified as *S. nigrum* while Mohy-ud-dint *et al*. (2010) postulate that *S. nigrum* complex includes *S. americanum* and *S. nigrum*. Therefore, an in-depth chemotaxonomic study of the literature about these plants should be undertaken. Also, genetic or environmental factors can influence alkaloid content, giving rise to a wide variability in the chemical composition of plants of the same species. This occurs in many crops, where sweet and bitter varieties can be differentiated among the same species. Another example is the fruits and roots of the Cucurbitaceae family, which may also contain cucurbitacins, which have lead to some cases of intoxication with bitter zucchini fruits in America and Australia. *Momordica* spp., whose fruits and leaves are appreciated for food consumption in Africa and Asia, may also have either bitter or nonbitter fruits with the presence of momordicosides (Gry *et al*. 2006).

The Apiaceae family is also important from the point of view of the correct identification of its members. Several species of this family are widely eaten throughout the world: *Daucus carota* L. (root, fruits), *Angelica sylvestris* L. (stems, fruits), *Apium*

graveolens L. (leaves, young stems), *Petroselinum* spp. (leaves, young stems), *Foeniculum vulgare* Mill. (leaves, young stems, fruits), *Pimpinella anisum* L. (fruits), *Pastinaca sativa* L. (leaves), and *Chaerophyllum aromaticum* L. (leaves). However, some of these vegetables may resemble other extremely toxic species, such as *Conium maculatum*, *Aethusa cynapium* L. (containing the lethal piperidine alkaloids coniine and conicein, respectively), *Cicuta virosa* L. or *Oenanthe crocata* L. (containing the very toxic polyacetylenes conicein and oenanthotoxin, respectively); they have been responsible for several deaths in recent decades, caused by erroneous identification of these species (Bruneton 2005).

Other potentially toxic compounds are not a problem for most people in the amounts found in edible plants, but may be particularly harmful for particular individuals, such as oxalates being both an antinutrient (forming a nonabsorbable Ca complex) but also provoking renal calculus, a concern for people suffering from renal disorders; the β-glucosides, vicin and convicin, causing favism in people with a genetic deficiency of glucose-6-phosphate-dehydrogenase; or nitrates causing metahemoglobinemia in infants. Many of these substances may also occur cultivated vegetables, acting as oxalate or nitrate accumulators, such as spinach or beet (*Spinacia* sp., *Beta* sp.) leaves.

Risks related to wild plant consumption may also arise because of the wrong use of some plant species, sold without control by health authorities or wrongly used in food supplements, or even medicines. For this reason, in many countries, health authorities are making efforts to regulate the use of plants in commercial preparations to promote consumer safety. For example, in 2009 the European Food Safety Authority (EFSA) published a compendium of botanicals that require specific attention while assessing the safety of products containing those species, due to previous reports confirming toxic, addictive, psychotropic or other substances of concern (European Food Safety Authority 2009). This publication is aimed to guide their use in food supplements, and requires correct interpretation. It should not to be considered as a list of toxic plants; in fact, it includes many species widely used as food all over the world, such as lemons (*Citrus limon* (L.) Burm. f) and

peaches (*Prunus persica* (L.) Batsch). This does not mean that they are toxic as eaten, but that some substance of concern may have been described in some parts of the plant, being harmful in small or large quantities. These quantities may sometimes significantly exceed what an adult can eat, but could easily be reached if an extract of the plant is included as an ingredient in a food supplement or pharmaceutical preparation. A good example would be nutmeg (*Myristica fragrans* Houtt.), widely used in gastronomy as a spice for its pleasant flavor; however, high doses of nutmeg or its extract (containing miristicin, elemicin, and safrol) are toxic for humans.

Advances in communication technology may sometimes contribute to sending the wrong messages to the population; nowadays, people receive high levels information without having a clear idea of what is reliable or not. So, besides health authority surveillance and recommendations, to avoid health problems derived from the incorrect use of wild plants, some popular myths should be discarded. People should understand that "natural" is not a synonym for "safe" and that the recovery of lost knowledge about wild edible plants is not an easy task when the knowledge chain has been broken for generations.

Being conscious of these concerns, the lost knowledge could be safely recovered through several strategies:

- education programs for children and adults about identification and handling of wild edibles and the risks of incorrect handling
- making people conscious that some species should only be used as medicines under the control of health professionals
- compilation of inventories of traditional knowledge relative to wild plants; these and other strategies have been sometimes conducted in some rural areas and funded by some governments perceiving the importance of this cultural heritage (Pardo de Santayana *et al*. 2014).

7.4 Conclusion

Taking into account the warnings regarding misidentification of

species or improper use, the revalorization of these traditional foods should be encouraged, as they represent valuable sources of nutrients, often lacking in many societies, and also contribute to diet diversification and preservation of the traditional knowledge, food habits, and identity of each geographical area.

Some species are widely spread and consumed as leafy vegetables in many different parts of the world, such as purslane (*Portulaca oleracea*), fat hen (*Chenopodium album*), nettles (*Urtica dioica*, *U. urens*), watercress (*Nasturtium officinale*), and spinach (*Spinacia oleracea*). Also the leaves of *Rumex* spp., *Sonchus* spp., *Mentha* spp., and *Brassica* spp., with differences in the species growing in each environment, are traditionally eaten in most places and cultures. Some other wild vegetables such as *Amaranthus* spp., *Centella asiatica* or different species of *Cucurbita*, often eaten in tropical Africa, America and Asia, are also widely used species.

These vegetables, among other species characteristic of specific geographical areas, are a valuable tool to improve the health status of populations by providing fiber, vitamins (folate, vitamin C, provitamin A), and minerals (K, Ca Fe, Mn in some cases), as well as other bioactive compounds such as antioxidants, for the human diet, in both developed or developing countries. Food-based strategies devoted to revalorizing these vegetables, driven by nongovernmental organizations and other local institutions, should be encouraged, including promoting gathering of autochthonous plants from the wild; acquisition of skills for their cultivation in home gardens (both at a very minimal cost), or improving bioavailability of nutrients by home preservation and preparation of food. Nutrition education should always be a complementary activity to ensure the effectiveness of these food-based approaches.

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8
Nutrients and Bioactive Compounds in Wild Fruits Through Different Continents

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8.1 Introduction

In many global nutritional and ethnobotanical studies, the nutritional role and health uses of wild edible plants have been reported and they often contain higher amount of nutrients and bioactive compounds than many cultivated species (Ruiz-Rodríguez *et al*. 2014a; Tardío *et al*. 2006; Trichopoulou *et al*. 2000). Wild edible plants significantly contribute to the diet of rural regions, being consumed during the year in fresh or processed forms (Leonti *et al*. 2006; Tardío *et al*. 2006). In recent years, bioactive compounds in underutilized plant foods have received attention due to their biological properties and benefits to human health, which include lowering the risk of cardiovascular disease, cancer, and other disorders associated with the aging process (Guerrero *et al*. 2010; Vasco *et al*. 2009).

In this scenario, wild fruits can also be considered as interesting high-value sources of nutrients and bioactive compounds with antioxidant activity, which could provide the basis for nutraceuticals, dietary and food supplements or functional foods (Heinrich *et al*. 2006). There is increasing interest in the phytochemical compounds in these traditionally used fruits. Fruits are generally recognized as essential for health, since human health depends to a large extent on factors such as high fruit and vegetable consumption (Trichopoulou *et al*. 2003). Deficiencies of essential micronutrients can increase the risk of illness or death from infectious diseases by reducing immune and nonimmune

defenses and by compromising normal physiology and development. Such nutrient deficiencies are widespread in low- and middle-income countries where wild fruits are a source of these compounds, with values close to or even higher than other cultivated fruits.

As we have already seen in Chapter 7, in every continent wild edible plants used are different due to the climatic situation and the socioeconomic context that will determine use and implications for human health.

8.2 African Wild Fruits as a Source of Nutrients and Bioactive Compounds

The socioeconomic development, historical circumstances, and geographic and climatic conditions of Africa have strongly influenced local food habits. In African countries, the utilization and marketing of indigenous plants have been central to the maintenance of the majority of rural communities. The FAO (1999) indicates that a great number of wild-collected species are used as food.

Rural populations, for instance in central and western parts of Sudan, often rely on wild species to meet their food and energy needs (Saied *et al*. 2008). A wide range of wild fruits used in Africa have the potential to provide rural households with their nutritional needs, such as fruits of *Sclerocarya birrea* Hochst. and *Adansonia digitata* L. (Ekesa *et al*. 2009).

Indigenous fruits play a very important role in the maintenance of rural people, especially for those living in dry areas (von Maydell 1989), where crop failure often results in poor nutrition (Maxwell 1991).

Despite these ancient practices, many African autochthonous plants are considered as neglected or underutilized in favor of other nonnative plants. However, they are known to have important uses at the local or national level, being part of

agriculture and food procurement systems and are important genetic resources to maintain biodiversity (Hammer *et al*. 2001; Grivetti & Ogle 2000). Local people utilize such indigenous plants for food, and they provide additional income generation through livestock feed, folk medicine, energy, and for their role in soil conservation such as the stabilization of sand dunes (Gebauer *et al*. 2007). For example, the use of wild food plants among the people of Ngai and Otwal was reported to be mainly due to the fact that these plants are perceived to be nutritional. They also contribute to food security in times of food shortage/famine. However, there was a reported decline in the use of wild food plants among the locals, the reasons being mainly seasonality of the plants and lack of time to collect these plants from the wild (Acipa *et al*. 2013). According to Odhav *et al*. (2007), the decrease in the use of indigenous plant sources by many African communities has resulted in poor diets and increased incidence of nutritional deficiency disorders.

The main nutritional problem in Africa is malnutrition, affecting around 200 million people, sub-Saharan Africa being the place with the highest prevalence of malnutrition in the world (Lopriore & Muehlhoff 2003). In Africa, this manifests as protein-energy malnutrition, but also as vitamin and mineral deficiencies. According to the WHO (2009), Africa shows the highest prevalence of anemia and vitamin A deficiency in the world and for micronutrient deficiencies, iron and iodine deficiencies are moderate-to-severe public health problems in most African countries, mainly in the central part of the continent, where almost half the population are affected by one of these deficiencies (Aguayo 2005; Sifri *et al*. 2003). A study performed by Allen *et al*. (2006) presented survey data suggesting that deficiencies of zinc, calcium, folate or vitamin D make a substantial contribution to African disease levels. In some areas of Africa, dietary fiber intake is also limited, mainly potentiated by the migration of communities from rural areas to cities, often inducing a diet with higher sugar and fat and low dietary fiber content (Ruel *et al*. 2005).

The existence of scientific knowledge about the nutrient composition of wild fruits used by indigenous African populations

is limited due to their socioeconomic situation. Analysis of the chemical composition of African wild edible fruits or medicinal species, in terms of nutrients, bioactive compounds or pharmacological activities, has only recently been undertaken. Many of the reports about bioactive compounds are mainly focused on the medicinal properties of these wild fruits, searching for medicinal applications of wild plants, rather than as food plants, searching for biological/pharmacological properties such as antimicrobial, antioxidant or antiinflamatory actions.

Some wild fruits such as *Adansonia digitata* (baobab fruits) are a very important food source and widely distributed throughout the continent. The importance of baobab to human livelihood is reviewed by several authors such as Kamatou *et al*. (2011), Kalinganire *et al*. (2007), and the FAO (1982). In addition, according to de Smedt *et al*. (2011), baobab is currently a crop of high international economic value since this fruit pulp has been approved for sale in the EU (EU Commission Decision 2008) and the USA (FDA 2008). Other species frequently consumed in the north of Africa include the dried fruits of *Ziziphus* spp., which are commonly ground into flour for bread production (Nassif & Tanji 2013), and fruits of *Balanites aegyptiaca* L., which are widely used during the dry season (Lockett *et al*. 2000).

In order to develop this work we have selected commonly consumed species of edible wild fruits. At least 30 different wild African fruits were reviewed regarding their nutritional and phytochemical composition. Studies on the chemical composition of African wild and indigenous fruit in different countries have been published, such as Burkina Faso (Glew *et al*. 1997; Lockett *et al*. 2000), Nigeria (Cook *et al*. 2000), Tanzania (Murray *et al*. 2001), Mozambique (Magaia *et al*. 2013), Zimbabwe (Nyanga *et al*. 2013), and Sudan (Saied *et al*. 2008).

Table 8.1 Macronutrient content and moisture (g/100 g dry weight) in wild edible fruits from Africa.

Species	Energy (kcal/100 g)	Moisture	TAC	Dietary fiber	Protein	Lipids	Ash	References
Adansonia	203–	4.7–	46.6–	5.7–	1.7–	0.2–	2.9–	Lockett

digitata L.	327	10.55	89.1	45.1	17	15.5	7.4	*et al*. 2000; Assogbadjo *et al*. 2012; Wehmeyer 1966, Osman 2004; Saka, & Msonthi 1994; Murray *et al*. 2001; Sidibe *et al*. 2002; Glew *et al*. 1997; Magaia *et al*. 2013; Stadlmary *et al*. 2013
Afraegle paniculata Engl.		5.91	76.86	9.12	4.19	0.33	3.59	Lockett *et al*. 2000
Balanites aegyptiaca L.	307	10.10	73.29	5.83	7.88	1.34	7.421	Lockett *et al*. 2000
Englerophytum magalismontanum (Sonder)		77.7	19.2	1.3	0.9	0.4	0.5	Wehmeyer 1966

T.D.Penn.								
Borassus aethiopum Mart.	–	7.08	64.07	18.60	4.25	1.74	4.26	Lockett *et al*. 2000
Bridelia ferruginea Benth	–	7.72	44.81	28.12	7.31	8.91	3.12	Lockett *et al*. 2000
Carissa macrocarpa Eckl.	–	79.7	16.4	1.6	0.5	1.1	0.7	Wehmeyer 1966
Coccinia sessilifolia (Sond.) Cogn.	–	82.3	12.7	1.3	2.1	0.2	1.4	Wehmeyer 1966
Dovyalis caffra Hook. f.	–	85.9	4.7	0.3	0.4	0.4	0.3	Wehmeyer 1966
Chrysophyllum albidum G.Don	116.5–122.6	70.8–76.3[a]	8–10.4[a]	3.5–4.5[a]	3.9–6.42[a]	2.6–5.6[a]	2.2–2.4[a]	Adepoju *et al*. 2012; Okerulu *et al*. 2015
Detarium microcarpum Guill. & Perr.	–	7.21	58.77	26.28	2.93	1.57	3.23	Lockett *et al*. 2000
Ficus sycomorus L.	–	87.47	41.47	34.77	7.50	7.09	9.28	Lockett *et al*. 2000
Gardenia aqualla Stapf & Hutch.	–	92.86	57.34	30.24	5.26	2.14	5.02	Lockett *et al*. 2000

Lannea schimperi Engl.	–	94.42	43.80	43.44	7.76	0.76	4.23	Lockett *et al.* 2000
Landolphia capensis Oliv.	–	64	33.1	0.8	1	0.2	0.6	Wehmeyer 1966
Parinari curatellifolia Planch. ex Benth	–	92.72	28.27	58.77	7.22	1.53	4.22	Lockett *et al.* 2000
Parkia biglobosa (Jacq.) R.Br. ex G.Don	–	91	72.15	15.92–19.45	5.09–15.34	2.05–8.7	4.78–4.5	Lockett *et al.* 2000; Olujobi *et al.* 2012
Sclerocarya cafra S.	–	91.7	7	0.5	0.5	0.1	0.2	Wehmeyer 1966
Sclerocarya birrea Hochst.	232	83	–	37.7	1.4–5.6	0.2–13.5	3–7.8	Thiongo *et al.* 2000; Glew *et al.* 1997; Magaia *et al.* 2013; Murray *et al.* 2001
Strychnos pungens Soler.	–	66.8	21.4	8.6	1.6	0.7	0.9	Wehmeyer 1966
Strychnos spinosa	–	89.23	59.82	23.96	11.70	1.94	2.58	Lockett *et al.*

Lam.								2000
Vitex doniana Sweet	–	–	–	–	2.2	15	–	Glew *et al.* 1997
Ximenia americana L.	–	–	24.9	27.51	15.21	28.23	4.13	Lockett *et al.* 2000
Ximenia caffra Sond.	67.2	95.75	26.3	0.7	3.1	1.3	1.4	Wehmeyer 1966
Ziziphus mauritiana Lam.	–	95.4	79.5–83.2	4.9–26.40	2.42–8.7	0.8–1.62	3.53–4.3	Lockett *et al.* 2000; Nyanga *et al.* 2013
Ziziphus spina-christi (L.) Willd.	314	–	80.6	–	4.8	0.9	–	Saied *et al.* 2008

a fw (fresh weight).

S, sucrose; TAC, total available carbohydrates.

Table 8.2 Vitamins and oxalic acids content in African wild fruits (mg/100 g dry weight).

Species	**Provitamin A**	**Thiamine**	**Riboflavin**	**Vitamin B_6**	**Vitamin C**	**Oxalic acid**	**Reference**
Adansonia digitata L.	–	0.57	0.16	–	67.9–337	9.5	Wehmeyer, 1966, Eromosele *et al.* 1991; Umaru *et al.* 2007;

							Magaia *et al.* 2013
Annona senegalensis Pers.	–	–	–	–	105	–	Eromosele *et al.* 1991
Balanites aegyptiaca L.	–	–	–	–	89.6	14.50	Eromosele *et al.* 1991; Umaru *et al.* 2007
Borassus aethiopum Mart.	26.61–27.42 (mg carotene/100 g)[a]	–	–	–	134.8–171.3[a]	11.30	Umaru *et al.* 2007; Ali *et al.* 2010
Carissa macrocarpa Eckl.	–	0.08	0.08	–	74.1	–	Wehmeyer 1966
Coccinia sessilifolia (Sond.) Cogn.	538 UI[b]	0.19	0.13	–	24.5	–	Wehmeyer 1966
Dovyalis caffra Hook.f.	71.8 UI[b]	0.01	0.05	–	117	–	Wehmeyer 1966
Chrysophyllum albidum G.Don	0.34–0.35[c]	1.30–1.55[a]	0.8–0.97[a]	1.45–1.8[a]	86.8–99.6[a]	0.53–0.54[a]	Adepoju *et al.* 2012
Detarium macrocarpum Harms	–	–	–	–	29.9	13.50	Eromosele *et al.* 1991; Umaru *et al.* 2007

Diospyros mespiliformis Hochst. ex A.DC.	–	–	–	–	–	12.20	Umaru *et al.* 2007
Haematostaphis barteri Hook.f.	–	–	–	–	26.7	6.30	Umaru *et al.* 2007; Eromosele *et al.* 1991
Hyphaene thebaica Mart.	–	–	–	–	–	13.50	Umaru *et al.* 2007
Landolphia capensis Oliv.	–	0.03	0.53	–	60.1	–	Wehmeyer 1966
Nauclea latifolia Blanco	–	–	–	–	–	2.22	Umaru *et al.* 2007
Parkia biglobosa (Jacq.) R.Br. ex G.Don	11.34–11.37[c]	100–120	20–30	–	–	11.10	Umaru *et al.* 2007; Olujobi *et al.* 2012
Phoenix dactylifera L.	–	–	–	–	–	6.90	Umaru *et al.* 2007
Sclerocarya birrea Hochst.	–	–	–	–	90–403.3	4.90	Umaru *et al.* 2007; Stadlmary *et al.* 2013; Thiongo *et al.*

							2000; Eromosele 1991; Lamien–Meda *et al. et al.* 2008; Magaia *et al.* 2013; Stadmary 2013
Sclerocarya cafra S.	–	0.03	0.05	–	67.9	–	Wehmeyer 1966
Strychnos pungens Soler.	84 IU β-carotene	0.10	0.74	–	21.9	–	Wehmeyer 1966
Vitex doniana Sweet	–	–	–	–	1.28–19.6	10.10	Umaru *et al.* 2007; Stadlmary *et al.* 2013; Eromosele 1991
Vitellaria paradoxa C.F.Gaertn.	–	–	–	–	–	7.02	Umaru *et al.* 2007
Ximenia americana L.	–	–	–	–	60.3	–	Eromosele *et al.* 1991
Ximenia caffra Sond.	369 IU β-carotene	0.04	0.04	–	22.5	–	Wehmeyer 1966
Ziziphus	–	–	–	–	2.8–	15.50	Umaru

mauritiana Lam.					43.8		*et al.* 2007; Stadlmary *et al.* 2013; Nyanga *et al.* 2013
Ziziphus spina-christi (L.) Willd.	–	–	–	–	30–98	16.20	Umaru *et al.* 2007; Eromosele *et al.* 1991; Saied *et al.* 2008

a fw (fresh weight);

b carotenoids;

c µg β-carotene/100 g.

IU, international units.

Table 8.3 Minerals (dry weight) in wild edible fruits from Africa: macroelements (mg/100 g) and microelements (µg/100 g; Fe mg/100 g).

	Microelements				Macroelements				
Species	**Fe**	**Cu**	**Mn**	**Zn**	**K**	**Na**	**Ca**	**Mg**	**Reference**
Adansonia digitata L.	0.017–4.40	550–6000	390–6000	0.01–2400	2308–2392	0.054–5.5	3.4–387	2.1–209.0	Lockett *et al.* 2000; Sidibe *et al.* 2002; Glew *et al.* 1997;

									Magaia *et al.* 2013; Eromosele *et al.* 1991
Annona senegalensis Pers.	1.33	170	430	640	–	–	28.9	42.2	Eromosele *et al.* 1991
Afraegle paniculata Engl.	12.80	1170	4900	450	–	–	914	96.87	Lockett *et al.* 2000
Balanite aegyptiaca L.	5.80	620	650	2920	–	–	120	81.37	Lockett *et al.* 2000
Borassus aethiopum Mart.	2.05–5.66[a]	720	290	190	–	–	44–108.3[a]	20.61–31.73[a]	Lockett *et al.* 2000; Ali *et al.* 2010
Bridelia ferruginea Benth	14.31	650	1410	1220	–	–	343	150	Lockett *et al.* 2000
Carissa macrocarpa Eckl.	0.56	210	–	–	298	1.58	22.6	19.5	Wehmeyer 1966
Coccinia sessilifolia (Sond.) Cogn.	0.2	200	–	–	685	4.9	37.9	2.2	Wehmeyer 1966
Dovyalis caffra Hook.F.	0.14	60	–	–	606	9.5	4.8	0.4	Wehmeyer 1966
Chrysophyllum albidum G.	2.23–2.29	5630–5820	4850–5160	8240–8270	666.2–700.8	35.5–54.5	365.5–425	–	Adepoju *et al.* 2012

Don									
Detarium microcarpum Guill. & Perr.	2.53	500	5030	780	–	–	15	97.07	Lockett *et al.* 2000
Ficus sycomorus L.	24.38	970	1470	2840	–	–	865	212	Lockett *et al.* 2000
Gardenia aqualla Stapf & Hutch.	3.36	840	920	570	–	–	526	182	Lockett *et al.* 2000
Landolphia capensis Oliv.	0.34	250	–	–	180	–	11.1	28.9	Wehmeyer 1966
Lannea schimperi Engl.	14.55	500	2780	1550	–	–	846	219	Lockett *et al.* 2000
Parinari curatellifolia Planch. ex Benth	5.08	330	640	2350	–	–	225	175	Lockett *et al.* 2000
Nauclea latifolia Blanco	1.8	150	210	920	–	–	42	70	Nkafamiya *et al.* 2006
Parkia biglobosa (Jacq.) R.Br. ex G. Don	0.74–3.1	488–970	3340–5440	1150–1260	1997.4	77.8	145.3–284	4.5–202	Lockett *et al.* 2000; Cook *et al.* 2000; Olujobi *et al.* 2012

Phoenix dactylifera L.	1.07	120	360	370	–	–	13	16.7	Eromosele *et al.* 1991
Sclerocarya birrea Hochst.	1.12–3	100	110	340–700	2753	15.2–30	36.2–481	138–310	Eromosele *et al.* 1991; Magaia *et al.* 2013; Glew *et al.* 1997
Strychnos pungens Soler.	0.91	150	–	–	5.10	2.60	30.3	26.2	Wehmeyer 1966
Strychnos spinosa Lam.	4.39	240	–	1080	–	–	130	141	Lockett *et al.* 2000
Vitex doniana Sweet	1.91	–	1140	–	–	–	139	124	Glew *et al.* 1997
Ximenia americana L.	1.97	170	510	630	–	–	3.3	25.3	Eromosele *et al.* 1991
Ximenia caffra Sond.	0.2	100	–	–	737	4.6	5.9	2	Wehmeyer 1966
Ziziphus mauritiana Lam.	17.85	6.3–1500	700–3500	600–1550	1865–2441	185–223	160–712.5	49.68–227	Eromosele *et al.* 1991; Lockett *et al.* 2000; Nyanga *et al.* 2013
Ziziphus	2.86–	640	610	1180	–	–	140–	90.7	Eromosele

spina-christi (L.) Willd.	-3						225.0		*et al.* 1991; Saied *et al.* 2008

[a] fw (fresh weight).

Table 8.4 Bioactive compounds (dry weight) in wild edible fruits from Africa: monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), tocopherols, and polyphenols.

Species	**Fatty acids (MUFA-PUFA; relative %)**	**Tocopherol α-tocopherol Micro/100 g**	**Total phenolic (mg GAE/100 g)**	**Phenolic acids (mg/100 g)**	**Flavonoids (mg QE/100 g)**	**Reference**
Adansonia digitata L.	18:3 (15%)	–	3518.33	–	31.70	Lamien-Meda *et al.* 2008; Glew *et al.* 1997
Balanites aegyptiaca L.	18:1 (22.47%)	–	–	–	–	Al Ashaal 2010
Borassus aethiopum Mart.	–	–	–	274.2–274.5	–	Ali *et al.* 2010
Chrysophyllum albidum G.Don	–	18.17–19.35	–	–	–	Adepoju *et al.* 2012
Dialium guineense Willd.	–	–	579.00	–	19.45	Lamien-Meda *et al. et al.* 2008
Diospyros mespiliformis	–	–	336.33	–	22.40	Lamien-Meda *et*

Hochst. ex A.DC.						*al*. 2008
Ficus sycomorus L.	–	–	190.58	–	24.15	Lamien-Meda *et al*. 2008
Gardenia erubescens Stapf & Hutch.	–	–	298.50	–	11.70	Lamien-Meda *et al*. 2008
Lannea microcarpa Engl. & K.Krause	–	–	240.58	–	23.35	Lamien-Meda *et al*. 2008
Parkia biglobosa (Jacq.) R.Br. ex G.Don	18:2 (0.24%)	–	380.92	–	–	Cook *et al*. 2000; Lamien-Meda *et al*. 2008
Sclerocarya birrea Hochst.	18:1 (32%)	–	226.2–505.83	–	33.90	Ndhlala *et al*. 2007; Lamien-Meda *et al*. 2008
Tamarindus indica L.	–	–	957.33	–	2.18	Lamien-Meda *et al*. 2008
Vitellaria paradoxa C.F.Gaertn.	–	–	381.67	–	20.70	Lamien-Meda *et al*. 2008
Ximenia americana L.	–	–	2239	–	30.95	Lamien-Meda *et al*. 2008
Ziziphus mauritiana	–	–	2352.50	–	56.88	Lamien-Meda *et*

Lam.	*al*. 2008

GA, gallic acid; QE, quercetin equivalent; TPC, total phenolic compounds.

The majority of the African wild fruits reviewed provide high moisture values (around 70–90%), except in some fruits (see Table 8.1), such as *Afraegle paniculata* Engl., *Borassus aethiopum* Mart., *Bridelia ferruginea* Benth., and *Detarium microcarpum* Guill. & Perr. African wild fruits have a energy value range around 67.2–327 kcal/100 g dry weight (dw) and proximal composition characterized by 4.7–89.1% of total available carbohydrates (TAC) content in dry weight values. Assogbadjo *et al*. (2012) reported values up to 89% of TAC for *Adansonia digitata* fruits; while some edible fruits particularly rich in carbohydrates can be found, such as *Ximenia americana* L., gathered in Nigeria (up to 25 g/100 g) (Lockett *et al*. 2000) or *Dovyalis caffra* Hook. f. (with lower values up to 5 g/100 g) (Wehmeyer 1966). Regarding the soluble sugar profile, sucrose is the main factor, as in fruits of *Adansonia digitata*, *Sclerocarya birrea* Hochst., and *Ziziphus spina-christi* (L.) Willd. Dietary fiber has been measured in several wild edible fruits and some of them have shown more than 3 g/100 g, which is used as the baseline to determine whether a food is rich in fiber (European Parliament and Council 2006); the majority of them presented more than 6 g/100 g, as in the case of *Sclerocarya birrea*, *Adansonia digitata*, and *Parinari curatellifolia* Planch. ex Benth., with values of 37.7, 45.1, and 58.7 g/100 g dw, respectively, soluble dietary fiber being the main fraction in *A. digitata* fruits (see Table 8.1). Commonly, in fruits, lipid content is below 1%, but the majority of the fruits present higher values, such as *Vitex doniana* Sweet and *Ximenia americana* fruits (values up to 28% according to Lockett *et al*. 2000). Moreover, in some cases, wild edible fruits may also present considerable protein content, up to 17% (Table 8.1).

Data on the vitamins and minerals in wild edible fruits traditionally consumed in Africa obtained from scientific literature are presented in Tables 8.2 and 8.3. Provitamin A is present in food plants, not as retinol, which is only naturally present in

animal tissues, but as carotenoids (α-carotene, β-carotene, and β-cryptoxanthin), mainly in their chloroplasts. this is biotransformed to retinol in the human body, demonstrating provitamin A activity after *in vivo* conversion (Britton *et al*. 1995; Ibrahim *et al*. 1991; Patton *et al*. 1990). Vitamin A activity is usually measured as retinol activity equivalents (RAE): 1 μg RAE = 1 μg of retinol = 12 μg β-carotene = 24 μg other provitamin A carotenoids (α-carotene or β-cryptoxanthin) (Mahan *et al*. 2013). In addition, most carotenoid compounds play an important role as dietary antioxidants; the daily intake of vitamin A for adults should be 0.5–1 mg of RAE to avoid vitamin A deficiency problems (Cuervo *et al*. 2009). Many wild fruits are richer sources of carotenoids, providing the whole amount of RAE needed for the human diet. An example is *Borassus aethiopum* Mart., which contains values up to 27.4 mg/100 g fresh weight (fw) of total carotenoids. Thus, the consumption of fresh wild fruits would be an excellent strategy to improve the nutritional quality of the African diet.

Moreover, many wild edible fruits, such as *Borassus aethiopum*, are notable for containing more than 100 mg/100 g of vitamin C, reaching quite remarkable values even up to 300–400 mg/100 g in *Sclerocarya birrea* and *A. digitata* fruits (Ali *et al*. 2010; Eromosele *et al*. 1991). *Ziziphus spina-christi* (L.) Willd. and *Ziziphus mauritiana* Lam. can also be considered as excellent sources of vitamin C. As can be seen in Table 8.2, the majority of these species could be a good source of vitamin C (providing at least 15% of nutrient reference value (NRV) (European Parliament and Council 2011)) or even considered as “high content of a vitamin” because they provide at least 30% of NRV) (European Parliament and Council 2011) with the consumption of just a 100 g portion of the wild fruit. Other vitamins studied in some African wild fruits were thiamine, riboflavin, and pyridoxine, with values between 0.01 and 120 mg/100 g dw in *Dovyalis caffra* Hook. f. and *Parkia biglobosa* (Jacq.) R. Br. ex G. Don, respectively.

Minerals can be divided from a nutritional point of view into two main groups (microelements and macroelements). In the reviewed fruits, the microelement content was comparable with average values found in common fruits. The iron content was around 2–6 times higher than the values for common fruits (see Table 8.3).

Ziziphus mauritiana and *Ficus sycomorus* presented the highest content but the latter may reach up to 24 mg Fe/100 g dw. *Adansonia digitata*, *Ziziphus mauritiana*, and *Parkia biglobosa* are notable for their Cu and Mn content, reaching up to 6 mg/100 g dw (Eromosele *et al*. 1991). The highest values for zinc concentration (>2.8 mg/100 g dw) were reported in fruits of *Balanites aegyptiaca* and *Ficus sycomorus* species.

Among macroelements, potassium is the main element in these wild fruits and calcium is one of the most important, since a deficiency in calcium intake in infants and the elderly leads to the development of skeletal health problems. Wild fruits such as *Z. mauritiana*, *A. digitata*, and *S. birrea* have high levels of K up to 2753 mg/100 g dw and *Ficus sycomorus* L. and *Lannea schimperi* Engl. have levels higher than 800 mg Ca/100 g of product, meaning that a 100 g portion of these wild fruits provides nearly 50% of the daily recommended levels of calcium for adults, and this ratio would be even higher for infants (Cuervo *et al*. 2009). Commonly, oxalic acid (see Table 8.2) may reduce calcium absorption by about one-sixth, so wild fruits with a ratio of oxalic acid/Ca lower than 2.5 are preferable for the human diet (Concon 1988; Derache 1990). All reviewed African wild fruits presented a good oxalic acid/Ca ratio (0.001–0.90), lower than 2.5. Even taking into account the presence of this antinutrient with the ability of complexing mineral elements, these wild fruit species may be considered as an interesting contribution to the African diet.

Wild fruits in Africa showed very low Na content (<20 mg/100 g dw), with the exception of *Chrysophyllum albidum* G. Don, *Z. mauritiana*, and *P. biglobosa* (Jacq.) R. Br. ex G. Don. Regarding other macroelements, Table 8.3 records data from different authors showing that magnesium is abundant in *S. birrea*, *Z. mauritiana*, and *Lannea schimperi* Engl.

According to Assogbadjo *et al*. (2012), *A. digitata* fruits are a good option for infant consumption to increase weight gain, being a good source of protein and fat, and are also an excellent source of calcium, iron, copper, and zinc (Tables 8.2 and 8.3).

Other bioactive compounds that are important in African wild fruits are monounsaturated fatty acids (MUFA), polyunsaturated

fatty acids (PUFA), and phenolic compounds (see Table 8.4). A high MUFA fraction was demonstrated in the mature fruits of *S. birrea*, mainly as oleic acid (up to 32%; 18:1n9), and *B. aegyptiaca* wild fruits (oleic acid up to 22.5%) (Al Ashaal *et al*. 2010; Glew *et al*. 1997), while *A. digitata* contained mainly *n*-6 PUFA as γ-linolenic acid (18:3n6), with values up to 15% (Glew *et al*. 1997). Moreover, *A. digitata* and *Z. mauritiana* fruits are very rich in phenolic compounds (3518.33 and 2352.50 mg gallic acid equivalent (GAE)/100 g dw, respectively (Lamien-Meda *et al*. 2008) and particularly in flavonoids with values up to 56.88 mg quercetin equivalent (QE)/100 g dw, such as in *Z. mauritiana* fruits.

More studies would be desirable to determine the amount of several important bioactive compounds, such as vitamins, individual phenolics and other phytochemicals, responsible for these actions in African wild fruits, which could be important in improving the antioxidant potential of African diets. Various relevant food strategies are increasing worldwide, which together with food fortification programs could help to reduce micronutrient malnutrition in Africa (Table 8.4).

8.3 American Wild Fruits as a Source of Nutrients and Bioactive Compounds

America is a good example of plant biodiversity all over the continent, due to the wide variety of climates (see Chapter 7). The economic, social, and cultural differences, from the countries in the north with a high degree of economic development to Central and South America, where areas with a better economic status are mixed with other depressed areas, are related to the importance of wild fruit in terms of consumption and nutritional/bioactive studies. In tropical countries, communities recognize and consume a wide variety of wild edible fruits; most are collected and eaten in rural areas (FAO 1990). A total of 71 different wild American fruits were reviewed regarding their nutritional and phytochemical composition; most were gathered in South America since the authors knowledge that there are very few studies pertaining to the

North American countries (Phillips *et al*. 2014).

The macronutrients, minerals, and bioactive compounds in wild American fruits have been reviewed. As expected, all these fruits provide high moisture values (around 70–90%), except some palm fruits (see Table 8.5), such as *Acrocomia aculeata* Lodd. ex Mart., *Euterpe edulis* Mart., *Syagrus oleracea* Mart. Becc., and *Syagrus romanzoffiana* (Cham.) Glassman. In terms of energetic values, *Acromia aculeata*, *Opuntia polyacantha* Haw., *Prunus americana* Marshall, and *Rubus idaeus* L. presented the highest levels (>100 kcal/100 g). In general, carbohydrates were the major macronutrient, with values up to 28.50 g/100 g (as reported by do Nascimento *et al*. (2011) in *Sideroxylon obtusifolium* (Roem. & Schult.) T.D. Penn. fruit) and up to 49.20 g/100 g in palm fruit (*Syagrus romanzoffiana*). In most cases, the wild fruits recorded in Table 8.5 could be considered as a good source of dietary fiber, highlighting species such as *Acrocomia aculeata*, *Prunus virginiana* L., *Rubus idaeus*, and *Syagrus* spp. (>20 g/100 g fw). Consumption of these wild fruits could be a good way to improve dietary fiber intake in American people, since 100 g of these wild fruits could provide at least 40% of dietary fiber recommendations (e.g. *Vasconcellea pulchra* (V.M. Badillo) V.M. Badillo) or up to 95% in *Rubus idaeus*. Wild American fruits are not particularly rich in proteins and lipids (see Table 8.5), except for palm fruits, which have a protein content up to 11.59 g/100 g fw (*Syagrus oleraceus*) and lipid content up to 44.08 g/100 g fw (*Euterpe edulis*).

Table 8.5 Macronutrient content (g/100 g fresh weight) in wild edible fruits from America.

Species	Energy (kcal/100 g)	Moisture	TAC	Dietary fiber	Protein	Lipids	Ash	Reference
Acrocomia aculeata Lodd. ex Mart.	285.65	5.98 – 34.32	35.06 – 36.22	11.14 – 20.26	2.76 – 6.72	14.93 – 28.94	1.78 – 2.17	Coimbra *et al*. 2011; Silva *et al*.

								2008
Annona crassiflora Mart.	90.47	76.05	10.3 – 12.78	4.82	0.4 – 1.22	2.37 – 3.83	0.58 – 1.37	Silva *et al.* 2008; Franco 1999
Averrhoa carambola L.	46	85	11.5	2	0.9	0.2	0.4	TACO 2006
Cavendishia bracteata (Ruiz & Pav. ex J. St.-Hil.) Hoerold	–	75.25 – 76.71	–	3.16 (pectin: 0.41 – 0.46)	0.98	0.1	0.66 – 0.71	Reyes *et al.* 2007
Cereus jamacaru DC	–	85.82 – 86.28	9.76 – 9.86	–	1.8 – 2.5	1.08– 1.98	0.43 – 0.64	do Nascimento *et al.* 2011
Clidemia rubra (Aubl.) G. Don f	–	–	–	8.85	1.18	1.03	–	Gordon *et al.* 2012
Campomanesia cambessedeana O. Berg.	47.36	87.31	10.57	1.54	0.50	0.12	0.04	Silva *et al.* 2008
Eugenia spp.	–	84.93	9.86	–	1.01	2.76	0.6	do Nascimento *et al.* 2011
Eugenia dysenterica	20.01 –	91.56 –	3.08 – 5.54	1.04 – 1.51	0.63 – 0.82	0.44 – 0.57	0.18 – 0.28	Silva *et al.*

D.C.	29.83	94.34						2008; de Morais Cardoso *et al*. 2011
Euterpe edulis Mart.	–	34.95 – 42.47	–	–	5.13 – 8.21	18.45 – 44.08	1.55 – 2.07	Borges *et al*. 2011
Euterpe precatoria Mart.	22 – 91	82.4 – 94.1	0.13 – 1.95	2.37 – 7.80	0.59 – 1.03	1.83 – 9.74	0.22 – 0.46	Yuyama *et al*. 2011
Euterpe oleracea Mart.	–	85.7	–	10.18 (SDF: 0.39;IDF: 9.79)	0.89	2.90	0.28	Rufino *et al*. 2011
Gaylussacia brasiliensis Meisn.	51	81.30	10.74	6.53	0.56	0.62	0.25	Bramoski *et al*. 2011
Hancornia speciosa Gomes	66.21	82.40	10.02	3.40	1.20	2.37	0.58	Silva *et al*. 2008
Hesperomeles ferruginea Lindl.	–	77.10 – 78.15	–	1.78 (pectin: 0.46 – 0.51)	0.49	0.1	0.67 – 0.69	Reyes *et al*. 2007
Hesperomeles obtusifolia (Pers.) Lindl.	–	75.22 – 76.44	–	3.06	0.62	0.14	0.76 – 0.84	Reyes *et al*. 2007
Macleania rupestris (Kunth) A.C.Sm.	–	83.00 – 83.05	–	2.65 (pectin: 0.44 – 0.47)	0.401	0.05	0.37 – 0.39	Reyes *et al*. 2007
Macleania salapa Benth.	–	83.57 – 83.64	–	3.07 (pectin: 0.52 –	0.57	0.1	0.38 – 0.40	Reyes *et al*. 2007

& Hook. f.				0.53)				
Mouriri pusa Gardner (Mart.) O. Berg	34.15	85.13	6.64	6.05	1.02	0.31	0.40	Silva *et al*. 2008
	58	81.3	15.3	2.3	0.6	0.1	0.4	do Nascimento *et al*. 2011
Opuntia ficus-indica (L.) Mill.	44.5	–	9.38	0.06	1.15	0.21	0.57	Souza *et al*. 2007
Opuntia leucotricha D.C.	–	–	15	13.5	4.5	3.2	–	García-Pedraza *et al*. 2005
Opuntia polyacantha Haw.	130	60.7	–	7.2 (IDF: 6.1; SDF: 1.1)	1.6	0.3	0.65	Barbosa *et al*. 2007
Pilosocereus gounellei (F.A.C.Weber ex K. Schum.) Byles & G.D. Rowley	–	77.45	15.83	1.47	2.65 – 5.47	3.16	0.91	do Nascimento *et al*. 2011
Pilosocereus pachycladus F. Ritter	–	85.89	8.72	–	2.10	2.66	0.63	do Nascimento *et al*. 2011;

								Barbosa *et al.* 2007
Prunus americana Marshall	157	76.68	–	8 (IDF: 7.4; SDF: 0.7)	2.6	0.4	0.8	Phillips *et al.* 2014
Prunus virginiana L.	162	60.72	13.65	20 (IDF: 18.5; SDF: 1.5)	3	1.7	0.93	Phillips *et al.* 2014
Psidium araca Raddi	37.09	82.36	7.67	8.65	0.5	0.49	0.33	Silva *et al.* 2008
Psidium schenckianum Kiaersk.		68.86	26.60	–	1.64	1.36	1.54	do Nascimento *et al.* 2011
Rosa pratincola Greene	32	58.66	–	4.8 (IDF: 4.5; SDF: 0.5)	3.2	0.1	1.18	Phillips *et al.* 2014
Rubus idaeus L.	162	84.48	–	24.1 (IDF: 21.2; SDF: 2.9)	1.6	0.3	0.28	Phillips *et al.* 2014
Sideroxylon obtusifolium (Roem. & Schult.) T.D. Penn.		57.39	28.50	–	2.86	9.62	1.23	do Nascimento *et al.* 2011

Syagrus oleracea (Mart.) Becc.		8.71	40.32	20.64	11.59	13.60	5.16	Coimbra *et al.* 2011
Syagrus romanzoffiana (Cham.) Glassman		7.75	49.20	26.98	5.41	7.68	3.21	Coimbra *et al.* 2011
Tacinga inamoena (K. Chum.) N.P. Taylor and Stuppy		82.60	14.27	–	0.97	1.23	0.93	do Nascimento *et al.* 2011
Talisia esculenta Radlk.	56.35	83.13	12.51	2.4	1.15	0.19	0.61	Silva *et al.* 2008
Vasconcella pulchra (V.M. Badillo) V.M. Badillo		83.15 – 83.87	4.19 – 4.92	9.88 – 9.91 (pectin: 0.02 – 0.05)	0.18 – 0.20	0.23 – 0.27	1.57 – 1.68	Matute & Tirado 2003
Vasconcella x heibornii V.M. Badillo		76.04 – 76.92	5.21 – 5.98	15.81 – 15.85 (pectin: 0.18 – 0.24)	0.18 – 0.24	0.18 – 0.24	1.57 – 1.69	Matute & Tirado 2003
Ziziphus joazeiro Mart.		76.11	19.38	–	2.19	1.11	1.21	do Nascimento *et al.* 2011

IDF, insoluble dietary fiber; SDF, soluble dietary fiber; TAC, total available carbohydrates.

Table 8.6 Vitamin content (fresh weight) in wild edible fruits from America.

Species	Provitamin A (REA; µg/100 g)	Vitamin B_9 (µg/100 g)	Vitamin B_6 (mg/100 g)	Vitamin C (mg/100 g)	Vitamin K (µg/100 g)	Niacin (mg)	Pantothenic acid (mg)	Riboflavin (mg)	Thiamine (mg)	Reference
Cavendishia bracteata (Ruiz & Pav. ex J. St.-Hil.) Hoerold	–	–	–	3.72 – 4.32 (AA)	–	–	–	–	–	Reyes *et al.* 2007
Clidemia rubra (Aubl.) G. Don f	–	–	–	8.44 (AA)	–	–	–	–	–	Gordon *et al.* 2012
Eugenia dysenterica D.C.	45.53	25.74	–	34.11 (AA: 30.03; DHA: 4.08)	–	–	–	–	–	de Morais Cardoso *et al.* 2011
Eugenia pyriformis Cambess. in A. St.-Hil.	141.6	–	–	39.3	–	–	–	–	–	Rufino *et al.* 2011
Euterpe edulis Mart.	158.3	–	–	186	–	–	–	–	–	Rufino *et al.* 2011

Euterpe oleracea Mart.	333.3	–	–	84	–	–	–	–	–	Rufino *et al.* 2011
Hesperomeles ferruginea Lindl.	–	–	–	15.21 – 18.31 (AA)	–	–	–	–	–	Reyes *et al.* 2007
Hesperomeles obtusifolia (Pers.) Lindl.	–	–	–	41.75 – 42.06 (AA)	–	–	–	–		Reyes *et al.* 2007
Macleania rupestris (Kunth.) A.C. Sm.	–	–	–	4.17 – 4.46 (AA)	–	–	–	–	–	Reyes *et al.* 2007
Macleania salapa Benth. & Hook. f	–	–	–	4.53 – 4.62 (AA)	–	–	–	–	–	Reyes *et al.* 2007
Malpighia emarginata ex D.C.	116.6	–	–	1357	–	–	–	–	–	Rufino *et al.* 2011
Mouriri guianensis Aubl.	391.6	–	–	27.5	–	–	–	–	–	Rufino *et al.* 2011
Myrciaria dubia (Kunth.) McVaugh	33.3	–	–	1882	–	–	–	–	–	Rufino *et al.* 2011
Myrciaria cauliflora (Mart.) O.	26.6	–	–	238	–	–	–	–	–	Rufino *et al.* 2011

Berg										
Opuntia polyacantha Haw.	–	–	0.079	11.3	2.9	0.293	0.06	<0.1	0.08	Phillips *et al.* 2014
Prunus americana Marshall	55.6	1.52	0.093	10.3	11.2	0.367	0.301	<0.1	0.005	Phillips *et al.* 2014
Prunus virginiana L.	97.5	3.92	0.198	1.0	21.1	0.628	0.398	0.173	0.034	Phillips *et al.* 2014
Rosa pratincola Greene	586.8	7.18	0.076	426	25.9	1.3	0.8	0.166	0.016	Phillips *et al.* 2014
Rubus idaeus L.	8	4.78	0.104	26.4	6.6	1.03	0.3	<0.1	0.018	Phillips *et al.* 2014

AA, ascorbic acid; DHA, dehydroascorbic acid; REA, retinol equivalent activity.

Table 8.7 Minerals (fresh weight) in wild edible fruits from America: macroelements (mg/100 g) and microelements (µg/100 g; Fe mg/100 g).

	Microelements				**Macroelements**				
Species	**Fe**	**Cu**	**Mn**	**Zn**	**K**	**Na**	**Ca**	**Mg**	**Reference**
Acca sellowiana (O. Berg) Burret	0.4	160	62	22	68.4	0.4	6.8	3.9	Kinupp & Barros 2008
Acrocomia aculeata Lodd. ex Mart.	0.88	–	–	–	–	–	2.15	–	Silva *et al.* 2008
Annona crassiflora Mart.	0.43	–	–	–	–	–	29	–	Silva *et al.* 2008

Averrhoa carambola L.	1.72	19	8	48	102	3	10	13	Leterme *et al.* 2006
Butia capitata (Mart.) Becc.	0.7	100	100	100	293.9	0.9	3	6.3	Kinupp & Barros 2008
Carioca papaya L.	37	1	3	9	85	7	16	10	Leterme *et al.* 2006
Clidemia rubra (Aubl.) G. Don f	1.73	10	9.61	63	163.42	0.85	43.62	9.21	Gordon *et al.* 2012
Compomanesia cambessedeana Berg.	0.24	–	–	–	–	–	8	–	Silva *et al.* 2008
Cyphomandra betacea (Cav.) Sendt	0.41	11	20	2	524	6	26	20	Leterme *et al.* 2006
Eugenia dysenterica D.C.	0.02	–	–	–	–	–	8	–	Silva *et al.* 2008
Eugenia involucrata D.C.	0.4	37	100	100	124.9	4.1	9.8	6.7	Kinupp & Barros 2008
Eugenia myrcianthes Nied.	0.2	19	100	100	112.4	0.3	5.1	7.2	Kinupp & Barros 2008
Eugenia malaccensis L.	0.15	3	6	7	164	10	15	25	Leterme *et al.* 2006
Eugenia	0.38	7	8	18	78	2	25	38	Leterme

stipitata McVaugh									*et al.* 2006
Eugenia uniflora L.	0.49	7	11	19	165	<0.1	48	38	Leterme *et al.* 2006
Euterpe precatoria Mart.	0.46–1.16	–	–	163.43–585.37	73.78–376.69	0.12–13.92	15.99–57.85	–	Yuyama *et al.* 2011
Gaylussacia brasiliensis Meisn.	6.03	100	–	400	115.40	13.21	58.23	21.90	Bramoski *et al.* 2011
Hancornia speciosa Gomes	0.88	–	–	–	–	–	35	–	Silva *et al.* 2008
Hylocereus triangularis (L.) Britt. & Rose	0.5	15	11	34	207	8	31	23	Leterme *et al.* 2006
Malpighia glabra L.	0.47	4	9	19	202	<0.1	38	56	Leterme *et al.* 2006
Morinda citrifolia L.	0.57	11	28	21	374	13	43	17	Leterme *et al.* 2006
Mouriri pusa Gardner	0.23	–	–	–	–	–	22.3	–	Silva *et al.* 2008
Myrciaria cauliflora (Mart.) O. Berg	0.33	6	28	19	213	5	22	16	Leterme *et al.* 2006
Opuntia leucotricha D.C.	–	–	–	–	3.277	12.1	–	–	García-Pedraza *et al.*

									2005
Opuntia polyacantha Haw.	1.150	100	1560	611	130	<9	180	69	Phillips *et al.* 2014
Passiflora edulis Sims	0.61–0.66	5–6	12–16	20–43	100 – 764	16–30	22 – 53	16 – 26	Leterme *et al.* 2006
Psidium araca Raddi	0.21	–	–	–	–	–	21	–	Silva *et al.* 2008
Psidium guajava L.	0.28	8	9	20	332 – 366	5 – 7	20 – 29	12 – 17	Leterme *et al.* 2006
Pilosocereus gounellei (F.A.C. Weber) Byles & G.D. Rowley		–	–	–	–	–	8	–	Barbosa *et al.* 2007
Prunus americana Marshall	0.174	35	76	94	364	<9	11	8	Phillips *et al.* 2014
Prunus virginiana *L.*	0.685	186	417	328	379	<9	60	27	Phillips *et al.* 2014
Rosa pratincola Greene	1.06	113	1.020	245	429	<9	169	69	Phillips *et al.* 2014
Rubus idaeus L.	1.150	100	1.560	611	130	<9	36	691	Phillips *et al.* 2014
Solanum sisymbrifolium Lam.	0.6	67	67	100	256.9	5.0	14.7	14.4	Gordon *et al.* 2012
Talisia	0.60	–	–	–	–	–	26.7	–	Silva

esculenta Radlk.									*et al.* 2008
Vasconcella pulcra (V.M. Badilio) V.M. Badillo	7.04 – 8.06	22 – 77	–	40 – 51.5	598.52 – 658.92	22.62 – 32.62	17.61 – 19.99	34.37 – 35.39	Matute & Tirado 2003
Vasconcella x heibornii V.M. Badillo	6.31 – 6.61	<0.5	–	83 – 156	371.09 – 417.94	6.31 – 6.61	13.51 – 18.24	31.01 – 35.57	Matute & Tirado 2003
Ziziphus jujuba Miller	3.37	10	13	66	107	4	385	11	Leterme *et al.* 2006

Table 8.8 Bioactive compounds (fresh weight) in wild edible fruits from America: polyunsaturated fatty acids (PUFA) and polyphenols.

Species	**Fatty acids (PUFA; relative %)**	**Total phenolics (mg GAE/100 g)**	**Total phenolic acids (mg/100 g)**	**Total flavonoids (mg QE/100 g)**	**Total anthocyanins (mg/100 g)**	**Reference**
Amomyrtus meli (Phil.) D. Legrand & Kausel	–	17.52	–	11.76	13.33	Ramírez *et al.* 2011
Berberis microphylla G. Forst.	–	65.53	–	45.72	51.62	Ramírez *et al.* 2011
Clidemia	–	–	Quercetin	–	Delphinidin	Gordon

rubra (Aubl.) G. Don f			3-*O*-arabinoside (5.26); quercetin 3-*O*-rhamnoside (5.06)		3-*O*-rutinoside (23.74); cyanidin 3-*O*-rutinoside (39.43)	*et al.* 2012
Eugenia spp.	–	–	–	16.85	–	do Nascimento *et al.* 2011
Eugenia calcyna	–	225	–		–	Rocha *et al.* 2011
Eugenia dysenterica D.C.	–	90 – 111	–	–	–	Rocha *et al.* 2011
Eugenia klostzchiana	–	212 – 217	–	–	–	Rocha *et al.* 2011
Eugenia punicifolia (Kunth.) D.C.	–	327	–	–	–	Rocha *et al.* 2011
Eugenia pyriformis Cambess. in A. St.-Hil.	–	127	–	17.5	1.13	Rufino *et al.* 2011
Euterpe edulis Mart.	18:1 (44.17 – 55.51%); 18:2 (18.19 – 25.36%)	755	Ferulic (1.48 – 8.16); gallic (7.95 – 52.25); protocatechuic (6.29 –	375; catechin (0.74 – 16.24), epicatechin (6.83 – 30.56); quercetin	192 ; cyanidin-3-glucoside (14.84 – 409.85)	Rufino *et al.* 2011; Borges *et al.* 2011

			33.06)	(17.59 – 36.34)		
Euterpe precatoria Mart.	18:1 (58.7 – 74.6); 18:2 (2.0 – 11.6); C18:3 (0.7 – 1.3)	–	*p*-Hydroxybenzoic acid; vanillic acid; ferulic acid; benzoic acid	Orienthin; catechin; epicatechin; proanthocyanidins	Cyanidin derivatives; peonidin – 3-O-glucoside (128.40 – 868.91)	Yuyama *et al.* 2011; Heinrich *et al.* 2011
Euterpe oleracea Mart.	18:1 (1.56 g/100 g), 18:2 (0.31 g/100 g)[a]	454	–	91.3	111	Rufino *et al.* 2011
Gaylussacia brasiliensis Meisn.	18:1 (16.5 g/100 g), 18:2 (33.4 g/100 g), 18:3 (28.8 g/100 g)[a]	417.81	–	–	Cyanidin 3-glucoside (240)	Bramoski *et al.* 2011
Luma chequen F. Phil.		5.11	–	2.57	1.54	Ramírez *et al.* 2011
Luma apiculata (D.C.) Burret	–	27.61	–	12.80	15.24	Ramírez *et al.* 2011

Malpighia emarginata ex. D.C.	–	1063	–	9.6	18.9	Rufino *et al.* 2011
Myrciaria dubia (Kunth.) McVaugh	–	1176	–	20.1	42.2	Rufino *et al.* 2011
Myrciaria cauliflora (Mart.) O. Berg	–	440	–	147	58.1	Rufino *et al.* 2011
Mouriri guianensis Aubl.	–	549	–	41	3.3	Rufino *et al.* 2011
Pilosocereus gounellei (F.A.C. Weber) Byles and G.D. Rowley	–	–	–	22.76	–	do Nascimento *et al.* 2011
Pilosocereus pachycladus F. Ritter	–	–	–	8.23	–	do Nascimento *et al.* 2011
Psidium schenckianum Kiaersk.	–	–	–	24.59	–	do Nascimento *et al.* 2011
Sideroxylon obtusifolium (Humh. Ex Roem. and Schult.)	–	–	–	55.99	Anthocyanins (56.69)	do Nascimento *et al.* 2011

T.D. Penn.						
Tacinga inamoena (K. Chum.) N.P. Taylor and Stuppy	–	–	–	10	–	do Nascimento *et al*. 2011
Ugni molinae Turcz.	–	9.24	–	5.54	6.85	Ramírez *et al*. 2011
Vaccinium corymbosum L.	–	45.86 mg GAE/g dw	–	18.50	Cyanidin 3-*O*-glucoside (21.41 dw)	Ramírez *et al*. 2011

[a] values expressed in mg/100 g;

dw, dry weight; GAE, gallic acid equivalents; QE, quercetin equivalent.

Data on relevant levels of vitamins and minerals in wild edible fruits traditionally consumed in America are presented in Tables 8.6 and 8.7. Wild American fruits contain a great variety of carotenoids as a source of provitamin A activity (see Table 8.6), with values that range between 45.53 and 391 µg/100 g fw in *Eugenia dysenterica* D.C. and *Mouriri guianensis* Aubl., respectively (de Morais Cardoso *et al*. 2011; Rufino *et al*. 2011). Other carotenoids evaluated were α-carotene, lutein, zeaxanthin, lycopene, and β-cryptoxanthin (Phillips *et al*. 2014). Vitamin C was the main hydrophilic vitamin present in wild American fruits, in particular *Myrciaria dubia* (Kunth.) McVaugh (1882 mg/100 g). Also, *Euterpe edulis*, *Myrciaria cauliflora* (Mart.), *Rosa pratincola*, and *Malpighiae marginata* ex D.C. stand up due to their high vitamin C content (186, 238, 426, and 1357 mg/100 g, respectively). In most cases, these wild fruits could be a good source of vitamin C.

Folic acid and folates (vitamin B_9) levels in some wild American fruits were also reported (de Morais Cardoso *et al*. 2011; Phillips *et al*. 2014). Some presented interesting values, as in the case of *Eugenia dysenterica* (25.74 µg/100 g fw). Other vitamins studied were niacin, pantothenic acids, riboflavin, thiamine, and vitamin K (also predominant in leafy vegetables; see Chapter 7).

Mineral (micro- and macroelements) content was reviewed in Table 8.7. Regarding microelements content, *Gaylussacia brasiliensis* Meisn., *Vasconcella* spp., and *Carica papaya* L. are notable for their high iron content, in all cases providing more than 15% of the iron recommended dietary allowance (RDA). *Acca sellowiana* (O. Berg) Burret, *Prunus virginiana*, and *Rosa pratincola* have a high copper content, and could provide 15% of RDA for this mineral (with values up to 150 µg/100 g), while *Rosa idaeus* and *Opuntia polyacantha* provide more than 15% RDA of manganese (up to 350 µg/100 g). Regarding zinc content, *Euterpe precatoria* Mart. and *Gaylussacia brasiliensis* Meisn. contain high levels. Potassium was the major macroelement reported in American wild fruits, in most cases with values higher than 300 mg/100 g fw, as in *Morinda citrifolia* L., *Cyphomandra betacea* Sendt, *Psidium guajava* L., *Prunus* spp., *Rosa pratincola*, and *Vasconcella* spp. In addition, *Opuntia polyacantha*, *R. pratincola*, and *Rubus idaeus* L. have high calcium levels (more than 150 mg/100 g of fresh fruit) (Phillips *et al*. 2014), so that 100 g of these fruits could provide more than 15% of the daily recommended levels of this mineral for adults (700–1000 mg/day) (Cuervo *et al*. 2009). *Opuntia polyacantha* and *R. pratincola* also have Mg content higher than 60 mg/100 g. As previously mentioned, wild fruits have a very low Na content (<20 mg/100 g), being remarkably low in *Acca sellowiana* (O. Berg) Burret, *Eugenia myrcianthes* Nied., *Eugenia uniflora* L., and *Malpighia glabra*, with values lower than 0.5 mg/100 g (Table 8.6 and 8.7).

Studies on fatty acid characterization in wild American fruits are scarce; only data about *Euterpe edulis*, *Euterpe predatoria*, *Euterpe oleracea*, and *Gaylussacia brasiliensis* were found (see Table 8.8). In *Euterpe* spp. oleic acid (18:1) was the

predominant MUFA followed by α-linoleic acid (18:2) and linolenic acid (18:3) as the principal PUFAs. Other bioactive compounds such as total phenolics, flavonoids, and anthocyanins are summarized in Table 8.8. Of the reviewed wild American fruits, *Malpighia emarginata* and *Myrciaria dubia* stand out due to their high total phenolic content (more than 1000 mg GAE/100 g). Other interesting wild fruits were *Mouriri guianensis* Aubl. and *Euterpe edulis*, the first one due to its high total phenolic content (549 mg GAE/100 g) and the second due to its high total phenolic (755 mg GAE/100 g), total flavonoid (375 mg/100 g) and anthocyanin content (192 mg/100 g), gallic acid, quercetin, and cyanidin-3-glucoside being reported as the main polyphenols in this wild fruit (Borges *et al*. 2011; Rufino *et al*. 2011) (Table 8.8).

8.4 Asian Wild Fruits as a Source of Nutrients and Bioactive Compounds

Asia is one of the most diverse continents with three major climatic realms: Siberia (north-east Asia), Monsoon (south-east Asia), and Desert (west and central Asia). This environmental variation promotes a wide vegetable biodiversity (see Chapter 7). Asian cuisine and medicine were fundamental to the traditional knowledge of plant uses, which in most cases are believed to share a common origin, such in Chinese and Indian tradition. Nevertheless, there is still an enormous amount of plant material which has not been studied and for which the nutritional composition is unknown. A total of 32 wild edible fruits have been reviewed via ethnobotanical surveys and other available information about nutritional and phytochemical composition.

Due to the economic situation in Pakistan and India and other developing countries, the main components of the diet of the diverse ethnic groups are wild plants, with wild fruits being an important source of macronutrients such as carbohydrates and important vitamins and minerals, essentials for normal human body physiology. Table 8.9 provides detailed information on the macronutrient composition in wild Asian fruits. In general, the

reviewed fruits presented an energy value and proximal composition close to cultivated fruits, with high moisture (55.11–92.67%) and carbohydrate content, in particular *Ziziphus rugosa* Lam. and *Aegle marmelos* Correa (20.7 and 29 g/100 g, respectively (Kubola *et al*. 2011; Mahapatra *et al*. 2012; Paul *et al*. 2013). In most cases, these fruits could be considered an important source of dietary fiber, with values up to 15.6 g/100 g as in *Antidesma velutinum* Blume (Judprasong *et al*. 2013). Lipid content is usually below 1 g/100 g, with some exceptions such as *Passiflora siamica* Craib (around 2 g/100 g) (Jin *et al*. 1999). In some cases, wild Asian fruits may present considerable protein content (up to 3 g/100 g), as reported by Mahapatra *et al*. (2012) in *Bridelia tomentosa* Blume and *Carissa spinarum* L.

Table 8.9 Macronutrient content (g/100 g fresh weight) in wild edible fruits from Asia.

Species	Moisture	TAC	Soluble sugars	Dietary fiber	Protein	Lipids	Ash	Reference
Aegle marmelos (L.) Correa	–	29	–	2.9 – 3.18	2.2	0.29	–	Kubola *et al*. 2011; Paul 2013
Antidesma velutinosum Blume	73.2	22.5	–	15.6	1.6	1.1	2.1	Judprasong *et al*. 2013
Averrhoa bilimbi L.	–	4	–	6.73	1.04	0.33	–	Paul 2013
Averrhoa carambola L.	92.67	–	4.55	0.62	–	0.24	–	Jin *et al*. 1999
Berberis lycium Royle	83.29	12.64	–	0.81	1.81	0.63	0.82	Stood *et al*. 2010
Bridelia tomentosa	78.54	16.26	15.75	–	3.17	–	–	Mahapatra *et al*.

Blume								2012
Carissa spinarum L.	73.2	12.43	8.37	–	3.64	–	–	Mahapatra *et al*. 2012
Cordia myxa L.	–	16	–	2	1.9	1	–	Paul 2013
Eugenia rothii Panigrahi	66.32	19.01	18	–	0.65	–	–	Mahapatra *et al*. 2012
Glycosmis pentaphylla Correa	61.7	4.3	1.35	–	0.8	–	–	Mahapatra *et al*. 2012
Litchi chinensis Sonn.	81.76	16.5	15.9	–	0.61	–	–	Mahapatra *et al*. 2012
Mimusops elengi L.	55.11	18.15	15.9	–	0.61	–	–	Mahapatra *et al*. 2012
Morinda tinctoria Noronha	78.34	4.86	4.3	–	0.23	–	–	Mahapatra *et al*. 2012
Morus alba L.	84.10	13.92 – 39	–	0.34 – 9.8	0.87 – 1.44	0.03 – 0.39	0.33	Sundriyal & Sundriyal 2001; Paul 2013
Passiflora indica L.	80.14	14.50	2.40	3.06	0.98	0.01	0.63	Sundriyal & Sundriyal 2001
Passiflora siamica Craib	80.80	–	10.27	0.11	–	2.4	–	Jin *et al*. 1999
Phyllanthus acidus	88.7	4.81	4.5	–	0.25	–	–	Mahapatra *et al*.

(L.) Skeels								2012
Phyllanthus emblica L.	80.3	18.7	–	2.4 – 6.1	0.5	–	0.5	Judprasong *et al.* 2013
Prunus cerasoides D. Don	83.00	14.29	1.18	1.24	0.59	0.1	0.53	Sundriyal & Sundriyal 2001
Rubus ellipticus Smith	78 – 80.6	8.22 – 10.14	0.44	6.29 – 15.3	0.77	1.37 – 1.6	0.79	Sundriyal & Sundriyal 2001; Jin *et al.* 1999
Solanum torvum Sw.	59.1	11.9	9.52	–	1.46	–	–	Mahapatra *et al.* 2012
Streblus taxoidies Kurz	84.61	4.75	1.31	–	0.68	–	–	Mahapatra *et al.* 2012
Spondias pinnata (L.f.) Kurz	81.9	16.5	–	0.9 – 7.5	0.4	0.1	1	Kubola *et al.* 2011; Judprasong *et al.* 2013
Terminalia chebula Retz	75.45	3.1	2.6	–	0.92	–	–	Mahapatra *et al.* 2012
Toddalia asiatica Baill.	63.47	7.2	7.2	–	1.15	–	–	Mahapatra *et al.* 2012
Ziziphus mauritiana Lamk.	79 – 87.4	15.49	11.5 – 12.9	0.79 – 2	0.46 – 2	0.47 – 1	–	Mahapatra *et al.* 2012;

								Paul 2013; Jin *et al.* 1999
Ziziphus oenophlia (L.) Mill	57.12 – 79.9	17.13	6.15 – 15.8	0.87	0.7	0.06	–	Mahapatra *et al.* 2012; Jin *et al.* 1999
Ziziphus rugosa Lam.	60.83	20.7	20.7	–	0.58	–	–	Mahapatra *et al.* 2012

TAC, total available carbohydrates.

The literature highlights the important deficiency in vitamin A and C in some developing Asian countries (see Chapter 7). Table 8.10 presents data regarding vitamin C and provitamin A content in Asian wild fruits. Vitamin A deficiency and age-related macular degeneration are accepted as serious public health problems in India (WHO 2000). In this respect, wild fruit consumption, for example *Artocarpus lacucha* Roxb., *Flacourtia jangomas* (Lour.) Raeusch, and *Garcinia mangostana* L., could prevent this deficiency due to their high β-carotene content expressed as provitamin A (see Table 8.10). *Flacourtia jangomas*, *Phyllanthus emblica* L., and *Prunus cerasoides* D. Don are notable for containing more than 200 mg of ascorbic acid/100 g fresh fruit, reaching almost 300 mg/100 g in *P. cerasoides* (Sundriyal & Sundriyal 2001). Thus, wild Asian fruits could provide at least 100% of this vitamin RDA.

Table 8.10 Vitamin content (fresh weight) in wild edible fruits from Asia.

Species	Provitamin A (REA; µg/100 g)	Vitamin C (mg/100 g)	Reference
Aegle	92 (IU)	65.6 – 77	Paul 2013;

marmelos (L.) Correa			Kubola *et al.* 2011
Antidesma velutinosum Blume	17.75	2	Judprasong *et al.* 2013
Artocarpus lacucha Roxb.	4609[a]	14	Shajib *et al.* 2013
Averrhoa bilimbi L.	61 (IU)	34.4	Paul 2013
Averrhoa carambola L.	–	16.48	Jin *et al.* 1999
Baccaurea ramiflora Lour.	218[a]	12.1	Shajib *et al.* 2013
Berberis lycium Royle	120	22.2	Stood *et al.* 2010
Eugenia rothii Panigrahi	92[b]	18.52	Mahapatra *et al.* 2012
Flacortia jangomas (Lour.) Raeusch.	1120[a]	256	Shajib *et al.* 2013
Garcinia mangostana L.	4230[a]	14.4	Shajib *et al.* 2013
Glycosmis pentaphylla Correa	17.23[b]	25.22	Mahapatra *et al.* 2012
Hibiscus sabdariffa L.	1232[a]	3.7	Shajib *et al.* 2013
Litchi chinensis Sonn.	–	7.2	Mahapatra *et al.* 2012
Mimusops elengi L.	88.52[b]	25.22	Mahapatra *et al.* 2012
Morinda tinctoria Noronha	27.4[b]	18.92	Mahapatra *et al.* 2012

Morus alba L.	–	286	Sundriyal & Sundriyal 2001
Passiflora indica L.	–	28	Sundriyal & Sundriyal 2001
Passiflora siamica Craib	–	8.61 – 15.72	Jin *et al.* 1999
Phyllanthus acidus (L.) Skeels	161[a]; 16.05[b]	20.8 – 36.7	Mahapatra *et al.* 2012; Shajib *et al.* 2013
Phyllanthus emblica L.	4.91	215 – 575	Kubola *et al.* 2011; Judprasong *et al.* 2013
Prunus cerasoides D. Don	–	319	Sundriyal & Sundriyal 2001
Rubus ellipticus Smith	–	4.10 – 11	Sundriyal & Sundriyal 2001; Jin *et al.* 1999
Solanum torvum Sw.	33.29[b]	37.4	Mahapatra *et al.* 2012
Streblus taxoides Kurz	21.97[b]	19.32	Mahapatra *et al.* 2012
Spondias pinnata (L.f) Kurz	3.83	37	Judprasong *et al.* 2013
Syzygium cumini (L.) Skeels	434[a]	14 – 25.7	Kubola *et al.* 2011; Shajib *et al.* 2013
Terminalia chebula Retz	64.46[b]	53.52	Mahapatra *et al.* 2012
Toddalia asiatica Baill.	139.49[b]	22.02	Mahapatra *et al.* 2012
Ziziphus mauritiana	16.72[b]	36.01 – 88	Mahapatra *et al.* 2012; Paul

Lamk.			2013; Jin *et al.* 1999
Ziziphus oenophia (L.) Mill	21.86[b]	17.65	Mahapatra *et al.* 2012; Jin *et al.* 1999
Ziziphus rugosa Lam.	13.91[b]	21.26	Mahapatra *et al.* 2012

[a] µg/100 g dry weight;

[b] mg β-carotene/g dry weight.

REA, retinol equivalent activity.

Regarding lipophilic vitamins, there is very little information about vitamin E content in wild Asian fruits. Vitamin E is the term used to designate related compounds, namely tocopherols and tocotrienols. The major isoform of this vitamin present in plant tissues is α-tocopherol, which is considered the most active form in humans, due to preferential absorption and distribution in the human body (Caretto *et al.* 2009). Wild fruits may be very good sources of vitamin E when compared with other wild plants. Judprasong *et al.* (2013) reported values of 0.16–0.96 mg/100 g of this vitamin in some Thai wild fruits (*Antidesma velutinum* Blume, *Phyllanthus emblica* L., and *Spondias pinnata* (L.f.) Kurz.).

Table 8.11 details the mineral composition in Asian wild fruits. Generally, these fruits are notable for their high mineral content. Iron was the main microelement, with values up to 12 mg/100 g in *Toddalia asiatica* Baill. (Mahapatra *et al.* 2012). This could provide 100% of the RDA for this micronutrient (9 mg/100 g), and could palliate anemia, which is considered as a public health problem (Nutrition Formulation 1982; WHO 2000). As previously mentioned for other wild fruits, as occurs in cultivated species, potassium was the main macroelement, with values up to 600 mg/100 g (reported in *Eugenia rothii* Panigrahi by Mahapatra *et al.* 2012). Other wild Asian fruits have high levels of other macroelements, such as calcium (*Mimusops elengi* L.), and microelements, such as copper (*Phyllanthus emblica*, *Ziziphus oenophia*, and *Aegle marmelos*) and zinc (*Glycosmis*

pentaphylla Correa and *Solanum torvum* Sw.).

Table 8.11 Mineral (fresh weight) content in wild edible fruits from Asia: macroelements (mg/100 g) and microelements (µg/100 g; Fe mg/100 g).

	Microelements				Macroelements				
Species	**Fe**	**Cu**	**Mn**	**Zn**	**K**	**Na**	**Ca**	**Mg**	**Reference**
Aegle marmelos (L.) Correa	0.82	751.3	75.31	160.3	158.7	–	26.08	6.19	Shajib *et al.* 2013
Antidesma velutinosum Blume	0.58	110	–	430	11	230	325	115	Judprasong *et al.* 2013
Artocarpus lacucha Roxb.	0.28	–	755.3	336.8	137.55	–	26.17	8.80	Shajib *et al.* 2013
Baccaurea ramiflora Lour.	0.14	23.56	103.7	85.78	18.81	–	4.95	10.73	Shajib *et al.* 2013
Berberis lycium Royle	2.61	–	–	–	161.42	–	–	14.5	Stood *et al.* 2010
Eugenia rothii Panigrahi	1.44	161.66	3128	457	676.63	8.64	20.47	–	Mahapatra *et al.* 2012
Flacourtia jangomas (Lour.) Raeusch.	0.07	29.79	268	865.6	17.61	–	5.10	10.37	Shajib *et al.* 2013
Garcinia mangostana L.	0.34	83.55	176.1	116.6	23.07	–	10.43	18.68	Shajib *et al.* 2013
Glycosmis pentaphylla Correa	2.02	725.56	1547	1129.85	258.82	11.88	34.03	–	Mahapatra *et al.* 2012
Litchi	0.05	25.53	238.9	297.31	31.19	0.18	0.91	–	Mahapatra

chinensis Sonn.									*et al.* 2012
Mimusops elengi L.	2.11	228.8	3.58	0.94	362.5	23.76	885.98	–	Mahapatra *et al.* 2012
Morinda tinctoria Noronha	0.5	145.12	1.16	0.31	78.81	3.70	10.64	–	Mahapatra *et al.* 2012
Phyllanthus acidus (L.) Skeels	1.48 – 1.86	200 – 489.91	1092 – 1913	0.8	104.4 – 302.44	8.96 – 10.8	11.3 – 22.43	–	Mahapatra *et al.* 2012; Shajib *et al.* 2013
Phyllanthus emblica L.	0.16	4	–	140	13	151	42	13	Judprasong *et al.* 2013
Solanum torvum Sw.	2.13	560	3072	1394.6	304.7	12.99	59.94	–	Mahapatra *et al.* 2012
Streblus taxoides Kurz	0.45	73.87	297	30.16	39.45	2.79	4.75	–	Mahapatra *et al.* 2012
Spondias pinnata (L.f) Kurz	0.22	3	–	12	13	163	189	45	Judprasong *et al.* 2013
Terminalia chebula Retz	0.76	275.45	247.2	1029	358.4	4.57	10.87	–	Mahapatra *et al.* 2012
Toddalia asiatica Baill.	12.26	12.04	213.2	49.27	218.63	10.02	24.99	–	Mahapatra *et al.* 2012
Ziziphus mauritiana Lamk.	0.42	70.04	214.2	201.8	41.20	2.07	6.48	–	Mahapatra *et al.* 2012; Paul

									2013; Jin *et al*. 1999
Ziziphus oenophia (L.) Mill	1.82	554.8	–	109.99	308.7	11.21	40.63	–	Mahapatra *et al*. 2012; Jin *et al*. 1999

Phenolic compounds also occur in Asian wild fruits (Table 8.12). *Phyllanthus emblica* and *Spondias pinnata* stand out due to their total phenolic content (3703 and 3178 mg GAE/100 g respectively), while *Diospyros decandra* Lour. was reported to have the highest total flavonoid content (187 mg/100 g). Regarding individual polyphenols, ferulic and caffeic acids were the main phenolic acids, while luteolin and quercetin were the main reported flavonoids (Kubola *et al*. 2011) (Tables 8.11 and 8.12).

Table 8.12 Bioactive compounds (fresh weight) in wild edible fruits from Asia: total phenolics, phenolic acids, and total flavonoids.

Species	**Total phenolics (mg GAE/100 g)**	**Total phenolic acids (mg/100 g)**	**Total flavonoids (mg 100 g)**	**Reference**
Aegle marmelos (L.) Correa	81.46[a]	–	21.68[b]	Kubola *et al*. 2011
Antidesma velutinosum Blume	973	Quercetin (3.3); caffeic acid (4.3); ferulic acid (3.0)	–	Judprasong *et al*. 2013
Carissa	1.80[a]	–	14.69[c]	Kubola *et*

carandas L.				*al*. 2011
Citrus daoxianensis S.W. He	43.46–45.38b	Caffeic acid (337 – 504 µg/g dw); ferulic acid (3467 – 5839 µg/g dw)	9.70 – 16.28 mg/g dw	Zhang *et al*. 2014
Citrus reticulata Blanco	29.38 – 51.14b	Vanillic acid (24 – 119 µg/g dw); caffeic acid (249 – 1256 µg/g dw); *p*-cumaric acid (198 – 834 µg/g dw); ferulic acid (1730 – 7780 µg/g dw)	7.95 – 20.66 mg/g dw	Zhang *et al*. 2014
Citrus unshiu Marc.	39.71b	*p*-Coumaric acid (154 µg/g dw); ferulic acid 81613 µg/g dw)	6.28 mg/g dw	Zhang *et al*. 2014
Citrus poonensis Hort. Ex Tanaka	36.54b	Vanillic acid (64 µg/g dw); caffeic acid (1273 µg/g dw); *p*-cumaric acid (416	10.59 mg/g dw	Zhang *et al*. 2014

		µg/g dw); ferulic acid (3322 µg/g dw)		
Chrysophyllum cainito L.	17.88[a]	–	11.17[c]	Kubola *et al*. 2011
Coccinia grandis (L.) Voigt	6.90[a]	–	3.71[c]	Kubola *et al*. 2011
Diospyros decandra Lour.	214.65[a]	Caffeic acid (100 mg/g dw); syringic acid (153 mg/g dw)	187.27[c]	Kubola *et al*. 2011
Elaeocarpus hygrophilus Kurz	11.67[a]	–	11.131[c]	Kubola *et al*. 2011
Ensete glauca Roxb.	1.27[a]	–	1.58[c]	Kubola *et al*. 2011
Flacortia indica (Burm.f.) Merr.	3.87[a]	–	4.30[c]	Kubola *et al*. 2011
Muntingia calabura L.	16.50[a]	Ferulic acid (378 mg/g dw)	9.30[c]	Kubola *et al*. 2011
Musa balbisiana Colla	20.61[a]	–	9.43[c] (rutin: 98.13 mg/g dw)	Kubola *et al*. 2011
Phyllanthus emblica L.	65.2[a]; 3703	Quercetin (2.6)	21.38[c]	Kubola *et al*. 2011; Judprasong *et al*. 2013
Pithecellobium dulce	3.85[a]	–	2.16[c]	Kubola *et al*. 2011

(Roxb.) Benth.				
Pouteria campechiana (Kunth.) Baehni	5.00[a]	–	4.58[c]	Kubola *et al*. 2011
Psidium guajava L.	10.80[a]	–	9.57[c]	Kubola *et al*. 2011
Spondias dulcis G.Forst.	2.90[a]	–	1.84[c]	Kubola *et al*. 2011
Spondias pinnata (L.f.) Kurz	46.78[a] ; 3178	Syringic acid (147 mg/g dw)	5.39[c]	Kubola *et al*. 2011; Judprasong *et al*. 2013
Syzygium cumini L. Skeel	4.97[a]	Gallic acid (417 mg/g dw); *p*-hydroxybenzoic acid (109 mg/g dw)	4.60[c] (luteolin: 164.33 mg/g dw)	Kubola *et al*. 2011
Terminalia chebula Retz.	14.03[a]	Protocatechin acid (216.69 mg/g dw); vanillic acid (124 mg/g dw); ferulic acid (246 mg/g dw)	12.69[c] (quercetin: 98.26 mg/g dw)	Kubola *et al*. 2011

[a] mg GAE/g dry weight;

[b] mg/g dry weight;

[c] mg RE/g dry weight.

dw, dry weight; GAE, gallic acid equivalents; RE, retinol equivalents.

8.5 European Wild Fruits as a Source of Nutrients and Bioactive Compounds

As for other continents, socioeconomic development, historical circumstances, geographic, and climatic conditions have influenced the food habits of Europe (see Chapter 7). Many wild fruits and herbs are still used in Europe to make homemade jams (e.g. *Sambucus nigra* L., *Rubus ulmifolius* Scott.), desserts, and spirits (e.g. *Arbutus unedo* L., *Prunus spinosa* L.), for direct personal consumption and also for sale.

According to ethnobotanical data recorded by Pardo de Santayana *et al*. (2007), some of the most popular wild fruits in southern Europe, in countries like Spain and Portugal, are those of the Rosaceae family such as *Rubus ulmifolius*, consumed as ripe berries or used for making liqueurs. In the Mediterranean area another important species is *Arbutus unedo*; the fruits of this small strawberry tree have been traditionally consumed raw as a snack, as a dessert or, sometimes, used for elaborating jam or liqueurs as reported by Bonet and Vallès (2002), Łuczaj *et al*. (2013), and Verde *et al*. (2003).

Other edible species include the wild fruits of *Prunus spinosa* and *Crataegus monogyna* Jacq., species that today have fallen somewhat into disuse but were widely consumed in the past, and especially in times of scarcity, along with other species such as *Sorbus* and *Rosa* (Tardío *et al*. 2006).

In this section we discuss a total of 14 wild fruits which have scientific reliable data regarding composition of nutrients and/or bioactive compounds. Some studies on the nutritional value of European wild fruits have been published, such as Doležal *et al*. (2001) in the Czech Republic and Jablonska-Rys *et al*. (2009) in Poland. In recent years some work has been conducted, principally in Mediterranean countries, focusing on the study of wild fruit composition, such as Fadda & Mulas (2010) in Italy, Ruiz-Rodríguez *et al*. (2011) and Morales *et al*. (2013) in Spain, Barros

et al. (2010) in Portugal, and Hacıseferoğulları *et al*. (2012) in Turkey.

Table 8.13 Macronutrient content (g/100 g fresh weight) in wild edible fruits from Europe.

Species	**Energy (kcal/100 g)**	**Moisture**	**TAC**	**Soluble sugars**	**Dietary fiber**	**Proteins**	**Lipids**	**Ash**	**References**
Arbutus unedo L.	98–210	42.7–72.1	16.9–37.9	8.02–28.1 (F)	12.6–19.9 (8.70–18.7 IDF)	0.65–1.62	0.23–1.02	0.25–1.37	Alarcão-E-Silva *et al*. 2001; Ayaz *et al*. 2000; Barros *et al*. 2010a; Ganhão *et al*. 2010; Morales *et al*. 2013; Özcan & Haciseferoğullari 2007; Ruiz-Rodríguez *et al*. 2011; Vidrih *et al*. 2013.
Crataegus	80–	32.1–	13.5–	1.54–	7.23–	0.39–	0.38–	1.07–	Barros

monogyna Jacq.	247	77.8	37.5	4.14 (F)	16.4 (5.50–15.3 IDF)	1.45	1.22	4.26	*et al.* 2011a; Boudraa *et al.* 2010; Dolezal *et al.* 2001; Egea *et al.*2010; Ganhão *et al.* 2010;Morales *et al.* 2013; Ruiz-Rodríguez 2014.
Fragaria vesca L.	–	–	87.33 [a]	2.1 (F)	–	2.21 [a]	2.2 [a]	8.21 [a]	Mikulic-Petkovsek *et al.* 2012
Myrtus communis L.	80–107	61.7–75.7	7.50–8.26	6.69–8.64 (total)	17.4	1.66–4.17	0.87–2.37	0.60–0.73	Aydın & Ozcan 2007; Díaz 2005; Fadda & Mulas 2010; Hacıseferoğulları *et al.* 2012. Mikulic-

									Petkovsek *et al*. 2012; Wannes *et al*. 2009; Wannes *et al*. 2010.
Prunus spinosa L.	61–190	43.1–70.8	8.64–35.5	4.95–14.9 (G)	11.2–13.4 (8.90–11.8 IDF)	0.08–3.08	0.30–0.90	1.09–3.39	Egea *et al*. 2010; Ertuk *et al*. 2009, Ganhão *et al*. 2010; Marakoğlu *et al*.2005, Morales *et al*. 2013; Pereira *et al* 2013; Ruiz-Rodríguez 2014; Ruiz-Rodríguez *et al*. 2014a
Rosa canina L.	398.37[a]	48.68[a]	93.16[a]	12.89[a] (F)	–	2.72[a]	0.65[a]	3.47[a]	Barros *et al*. 2010b;

									Mikulic-Petkovsek *et al*. (2012)
Rubus ulmifolius Scott.	51–145	57.8–83.8	8.28–23.8	2.35–17.8 (F)	7.05–16.0 (7.00–17.7 IDF)	0.56–2.32	0.19–0.63	0.60–1.1	Egea *et al*. 2010; Ganhão *et al*. 2010; Jabłońska-Ryś *et al*. 2009; Morales *et al*. 2013; Ruiz-Rodríguez 2014.
Rubus idaeus L.	–	–	–	2.6 (F)	–	–	–	–	Mikulic-Petkovsek *et al*. 2012; Çekiç & Özgen 2010.
Sorbus aucuparia L.	–	–	–	5.29 (G)	–	–	–	–	Mikulic-Petkovsek *et al*. 2012
Sambucus nigra L.	–	–	–	2.78 (F)	–	–	–	–	Mikulic-Petkovsek *et al*. 2012
Vaccinium	–	–	–	2.24–	–	–	–	–	Mikulic-

myrtillus L.				8.71 (F)					Petkovsek *et al*. 2012 Milivojevic *et al*.2012
Vaccinium vitis-idaea L.		–	–	3.7 (G)	–	–	–	–	Mikulic-Petkovsek *et al*. 2012
Ziziphus jujuba M.		66.10	33.93	4.29 (G)	5.011	2.67	1.15	1.23	Díaz 2005
Ziziphus lotus L.	55–65	12.3–12.3	–	9.25–10.5	4.29–5.42	0.82–1.54	0.77–0.81	2.81–3.35	Abdeddaim *et al*. 2014; Boudraa *et al*.2010; Mouni *et al*. 2012.

[a] dw, dry weight.

F, fructose; G, glucose; IDF, insoluble dietary fiber; S, sucrose; TAC, total available carbohydrates.

Table 8.14 Vitamin and organic acid content (fresh weight) in European wild edible fruits.

Species	Provitamin A (µg REA/100 g)	Vitamin B_9 (µg/100 g)	Vitamin C (mg/100 g)	Vitamin E (mg/100 g)	Organic acids (mg/100 g)	Reference
Arbutus unedo L.	1.5–45	–	6–431	0.13–9.72	–	Ganhão *et al*. 2010; Morales

						et al. 2013; Pallauf *et al.* 2008; Pereira *et al.* 2013; Ruiz-Rodríguez *et al.* 2011; Ruiz-Rodríguez *et al.* 2014a; Vidrih *et al.* 2013
Crataegus azarolus L.	–	–	21.99 AA	–	–	Egea *et al.* 2010
Crataegus monogyna Jacq.	90–130	–	9.62–38.5	3.17–3.55	146–608 (malic acid)	Boudraa *et al.* 2010; Dolezal *et al.* 2001; Egea *et al.* 2010; Morales *et al.* 2013; Ruiz-Rodríguez 2014;

						Ruiz-Rodríguez *et al*. 2014b
Dracaena draco L.	–	–	–	–	162.7 g/kg[b]	Silva *et al*. 2011
Myrtus communis L.	–	–	–	–	191–268 (citric acid)	Hacıseferoğulları *et al*. 2012
Prunus spinosa L.	23–37	–	2–30.7	5.19–5.61	249–333 (malic acid)	Barros *et al*. 2010a; Egea *et al*. 2010; Ganhão *et al*. 2010; Marakoğlu *et al*. 2005; Pereira *et al*. 2013.
Rosa canina L.	1150	–	27.49 AA; 213.8[a]–1252.37 vitC	–	12.80 g/kg (citric acid)	Barros *et al*. 2011b ; Egea *et al*. 2010; Mikulic-Petkovsek *et al*.2012; Dolezal *et al*. 2001

Rubus ulmifolius Scott.	30–33	–	10–33.8	6.17–15,91	66.1–130 (oxalic acid)	Jabłońska-Ryś *et al.* 2009; Egea *et al.* 2010; Morales *et al.* 2013; Ruiz-Rodríguez *et al.* 2014a
Rubus idaeus L.	–	–	22.34–45.00	–	–	Purgar *et al.* 2012
Sambucus nigra L.	–	–	116.70	–	9.4 g/kg (citric acid)	Jabłońska-Ryś *et al.* 2009; Mikulic-Petkovsek *et al.* 2012
Sorbus aucuparia L.	473.3	–	68.18	–	30.28 g/kg (malic acid)	Mikulic-Petkovsek *et al.* 2012; Jabłońska-Ryś *et al.* 2009; Dolezal *et al.* 2001
Sorbus	–	–	22.65	–	–	Egea *et al*

domestica L.			AA			*al*. 2010
Vaccinium myrtillus L.		–	60.11	–	5.7–23 g/kg (citric acid)	Jabłońska-Ryś *et al*. 2009
Vaccinium vitis-idaea L.		–	–	–	20 g/kg	Mikulic-Petkovsek *et al*. 2012
Ziziphus jujuba M.	22.4 β-Carotene	–	7.87	–	–	Díaz 2005
Ziziphus lotus L.	1.47–62.8 Carotenoids	–	5.67–167	0.97–9.85	–	Benammar *et al*. 2010; Boudraa *et al*. 2010

a dw, dry weight;

b aqueous extract.

AA, ascorbic acid; REA, retinol equivalent activity; VitC, vitamin C.

Table 8.15 Mineral content (fresh weight) in wild edible fruits from Europe: macroelements (mg/100 g) and microelements (μg/100 g; Fe mg/100 g).

	Microelements				**Macroelements**				
Species	**Fe (mg)**	**Cu (μg)**	**Mn (μg)**	**Zn (μg)**	**K (mg)**	**Na (mg)**	**Ca (mg)**	**Mg (mg)**	**Reference**
Arbutus unedo L.	0.38–1.47	57–179	37–230	330–668	115–768	5.5–36.1	28.9–237	17.2–23.7	Özcan & Haciseferoğullari 2007; Ruiz-Rodríguez *et al*.

									2011; Vidrih *et al.* 2013
Crataegus monogyna Jacq.	0.43–2.66	139–233	107–1141	200–540	205–1113	3.14–8.70	115–273	26.7–111	Boudraa *et al.* 2010; Dolezal *et al.* 2001; Ruiz-Rodríguez 2014
Myrtus communis L.	1.60–2.56	248–310	723–1021	962–1053	478–549.9	77.7–81.0	122–163.2	36.8–52.1	Hacıseferoğulları *et al.* 2012
Prunus spinosa L.	0.26–1.98	102–360	77–171	160–529	216–590	5.62–66.6	32.6–67.8	11.4–29.9	Ertuk *et al.* 2009; Marakoğlu *et al.* 2005; Ruiz-Rodríguez *et al.* 2014b
Rubus ulmifolius Scott.	0.69–1.64	132–404	270–2850	279–539	122–270	6.8–57.2	45.6–111.2	16.8–69.2	Ruiz-Rodríguez 2014
Ziziphus jujuba M.	0.567	107	133	347	334.46	3.11	89.76	22.49	Díaz 2005
Ziziphus lotus L.	1.17–1.37	–	1900–2260	386–460	118–141	10.2–12.6	–	349–416	Abdeddaim *et al.* 2014; Boudraa *et al.*

									2010

[a] dw, dry weight.

Table 8.16 Bioactive compounds (fresh weight) in wild edible fruits from Europe: fatty acids, carotenoids, tocopherols, total phenolic compounds, phenolic acids, flavonols, flavonoids, and anthocyanins.

Species	Fatty acids (%)	Carotenoids (µg REA/100 g)	Tocopherols	Total phenolic compounds (mg/100 g)	Phenolic acids (mg/100 g)	Flavonols (mg/100 g)	Flavonoids (mg/100 g)	Anthocyanins (mg/100 g)	Reference
Arbutus unedo L.	α-linoleic acid (C18:3*n*-3, 31.2%–37.1%)	1.5–45	0.02–8.93	367–639 GA	78.3–78.7[b]	–	0.57–1.48[b] 10.86 (quercetin 3–*O*-glucoside: 2.34 dw)	2.91–4.63[b]	Alarcão-E-Silva *et al.*2001; Ayaz *et al.* 2000; a*et al.* 2010b; Ganhão *et al.* 2010; Guimares *et al.* 2013; Morales *et al.* 2013; Pallauf *et al.*2008; Ruiz-Rodríguez *et al.*

									2014a; Vidrih *et al*. 2013
Crataegus azarolus L.	–	0.58 mg/100 g (β-Carotene)	–	–	379.16 GA	–	–	–	Egea *et al*. 2010
Crataegus monogyna Jacq.	C18:3n3 (3.7%–16.4%)	90–130	2.74–3.02	312–2525 GA	245–689 GA	–	Rutin (32.3–327)	511.28 (cyanidin-3-*O*-glucoside: 483.89 ng/g dw; Pelargonidin-3-glucoside: 0.88-21.4	Boudraa *et al*. 2010; Dolezal *et al*. 2001; Egea *et al*. 2010; Ganhão *et al*. 2010; Morales *et al*. 2013; Pereira *et al*. 2013; Rodrigues *et al*. 2012; Ruiz-Rodríguez 2014; Ruiz-Rodríguez *et al*. 2014b
Dracaena	–	–	–	–	3505.3	–	–	–	Silva

draco L.					mg/kg[c]				*et al.* 2011
Myrtus communis L.	High MUFA (oleic acid, C18:1*n*-9; 6.49%-72.1%	–	–	531–2411[b]	14.4–60.1[b]	114–577 flavanois[b]	114–577[b]	–	Cakir 2004; Wannes *et al.* 2009; Barboni *et al.* 2010; Serce *et al.* 2010; Wannes *et al.* 2010; Hacıseferoğullar *et al.* 2012
Prunus spinosa L.	High MUFA (oleic acid; 61.2%–11.6%)	23–37	5.05–5.41	1584–3005[b]	544–912 GA	–	19.1–180 (quercetin-3-*O*-rutinoside: 15.63)	898–1964 (pelargonidin-3-*O*-glucoside)	Egea *et al.* 2010; Ganhão *et al.* 2010, Guimares *et al.* 2013; Marakoğlu *et al.* 2005; Pereira *et al.*2013, Ruiz-Rodríguez 2014;

										Ruiz-Rodríguez *et al.* 2014b
Rosa canina L.	PUFA (68.44%)[a]	18.07–497.8[a] mg/100 g (β-Carotene)	52.13[a]	149.4 mg GAE/g[a] 3217.28 mg/100 g	609.19 GA	–	–		5.50 (Taxifolin pentoside 0.180 dw)	Barros *et al.* 2010b; Barros *et al.* 2011b; Guimares *et al.* 2013; Mikulic-Petkovsek *et al.* 2012; Egea *et al.* 2010
Rubus ulmifolius Scott	PUFA (62.9%–63.9%)	30–33	1.92–4.84	247–951 GA	156–672 GA	–		4.2–84.7 (rutin)	68–214 (pelargonidin-3-*O*-glucoside)	Jabłońska-Ryś *et al.* (2009; Egea *et al.* 2010; Ganhão *et al.* 2010; Morales *et al.* 2013; Ruiz-Rodríguez

									et al. 2014a
Rubus idaeus L.	–	–	–	148.6–347.9 GAE 35.4–48.3	–	–	–	6.56–29.62 (cyanidin 3-*O*-sophoroside)	Çekiç & Özgen 2010 Gülçin *et al.* 2011 Purgar *et al.* 2012
Sambucus nigra L.	–	–	–	535.98	–	–	–	–	Mikulic-Petkovsek *et al.* 2012
Sorbus aucuparia L.	–	–	–	226.58	–	–	–	–	Mikulic-Petkovsek *et al.* 2012; Jabłońska-Ryś *et al.* 2009
Sorbus domestica L.	–	0.29 mg/100 g (β-Carotene)	–	–	8.99 GA	–	–	–	Egea *et al.* 2010
Ziziphus lotus L.	PUFA (36.0%–50.6%)	62.8–1.47 (β-Carotene)	–	8.26 GA	–	–	–	–	Benammar *et al.* 2010 ; Boudraa *et al.* 2010; Rsaissi *et*

								al. 2013
Vaccinium myrtillus L.	–	–	387–614	–	–	–	330–334 (cyanidin-3-*O*-glucoside)	Milivojevic *et al.* 2012 Mikulic-Petkovsek *et al.* 2012; Jabłońska-Ryś *et al.* 2009
Vaccinium vitis-idaea L.	–	–	2.0	–	–	–	–	Mikulic-Petkovsek *et al.* 2012

[a] dw, dry weight;

[b] expressed as sum of individual compounds;

[c] aqueous extract.

GA, gallic acid; GAE, gallic acid equivalent.

Wild fruits usually present a moisture, energy value, and proximal composition very close to cultivated fruits. European wild edible fruits have high moisture content (30–80%) with the exception of *Ziziphus lotus* L. They also demonstrate a variable total available carbohydrate content of 7.5–37.9%, highlighting *Ziziphus jujuba* M., *Crataegus monogyna*, and *Arbutus unedo* L. (Barros *et al.* 2010a,b; Díaz 2005). Regarding the soluble sugar profile, fructose and glucose are the main sugars in edible wild fruits, which are especially rich in fructose, as can be found in some fruits such as *Arbutus unedo* or *Rubus ulmifolius* (Ruiz-Rodríguez 2014).

Dietary fiber has been measured in several wild edible fruits and some have more than 3 g/100 g fw, which is used as a minimum to state that a food is rich in fiber (European Parliament and Council 2006); the majority of fruits contained more than 6 g/100 g, as in the case of *Arbutus unedo* and *Myrtus communis* L. (mainly as insoluble dietary fiber) (Ruiz-Rodríguez 2014), as can be seen in

Table 8.13. These species could contribute to improving the dietary fiber intake in European populations (dietary fiber daily intake recommended by international agencies is 25–30 g, 75% soluble fiber and 25% insoluble); this could help to achieve beneficial health effects, such as improving gastrointestinal health status (which improves glucose tolerance in diabetics and decreases plasma cholesterol, among others) and colon cancer prevention (FAO/WHO 2004; Meseguer *et al*. 2001; Trumbo *et al*. 2002; Yamada 1996). Dietary fiber and other carbohydrates are the main contributors to the energy value, ranging around 51–247 kcal/100 g fw (see Table 8.13).

Lipid content is usually below 1%, with some exceptions such as *Ziziphus jujuba* fruits (around 1.2% according to Díaz 2005). In some cases, wild edible fruits may demonstrate a considerable protein content, up to 4%.

Data on levels of vitamins and minerals in wild edible fruits traditionally consumed in Europe are presented in Tables 8.14 and 8.15. Wild fruits contain a great variety of carotenoids, responsible for their different colors, such as β-carotene giving mainly orange colors. Many wild fruits are rich sources of these compounds and can be considered a good source of vitamin A (European Parliament and Council 2011); *Crataegus monogyna*, *Sorbus aucuparia* L., and *Rosa canina* L. fruits can provide the whole amount of RAE (as β-carotene) needed daily for the human diet. Thus, the consumption of these fresh wild fruits would be an excellent strategy to improve the nutritional quality of the human diet.

Regarding vitamin E, wild fruits may be a very good source of vitamin E compared with other wild plants. *Arbutus unedo* and *Rubus ulmifolius* Scott. stand out from other Mediterranean wild fruits for their very high levels of tocopherols, as shown by the studies of Morales *et al*. (2013) and Barros *et al*. (2010).

It is known that wild fruits are the best sources of vitamin C. Many European wild edible fruits, such as *Ziziphus lotus* and *Sambucus nigra*, contain more than 100 mg ascorbic acid/100 g fresh fruit, reaching quite remarkable values even up to 400 mg/100 g in *Arbutus unedo* fruits (Ruiz Rodriguez *et al*. 2014a).

These wild fruits could be very good alternatives to conventional fruits for their vitamin C contribution; as can be seen in Table 8.14, many of these species can provide the whole RDA with just a 100 g portion. European Parliament and Council (2011) Regulation (EU) No 1169/2011 on the use of food information for consumer labeling purposes can be used in Europe as a reference to establish if a given food is a source of a given nutrient. In this context, a food can be claimed as a “source of a vitamin/mineral” if a 100 g portion can provide 15% or more of the reference labeling value. Furthermore, it could also be considered as “high content of a vitamin/mineral” if a 100 g portion can provide 30% or more of the NRV. In the case of vitamin C recommendations, at least 12 or 24 mg/100 g respectively should be provided to make claims for these values, and these levels could be achieved in many fruits and vegetables, either conventional or unusual.

Regarding other organic acids, wild fruits such as *Crataegus monogyna* and *Sambucus nigra* should be mentioned (see Table 8.14), with levels in 100 g fresh weight of 600 mg malic acid and 940 mg citric acid.

For minerals, *Prunus spinosa*, *Crataegus monogyna*, and *Myrtus communis* may reach almost 2.5 mg Fe/100 g, which is a similar value to those found in other vegetables traditionally considered as good iron sources (spinach around 2 4 mg/100 g, according to Souci *et al*. 2008). *Rubus ulmifolius* fruits are notable for their Cu and Mn content (up to 404 and 2850 μg/100 g, respectively) (Ruiz-Rodríguez 2014), while *Arbutus unedo* and *M. communis* demonstrate higher Zn content (with values up to 1053 mg/100 g in wild fruits from Turkey) (Hacıseferoğulları *et al*. 2012). Regarding macroelements in wild European fruits, potassium and calcium are the main elements in *A. unedo* and *C. monogyna*, and magnesium in *Ziziphus lotus*. Calcium is one of the most important macroelements, helping to maintain skeletal health, and wild fruits such as *C. monogyna* and *A. unedo* have levels higher than 200 mg/100 g of fresh product (see Table 8.15), meaning that a 100 g portion provides nearly 25% of the adult RDA and this ratio would be even higher for infants (Cuervo *et al*. 2009).

Oxalic acid may reduce calcium absorption so wild fruits with a

ratio of oxalic acid/Ca lower than 2.5 are preferable for the human diet (Concon 1988; Derache 1990), as can be seen in C. *monogyna*, *R. ulmifolius*, *A. unedo*, and *Prunus spinosa* (with ratios around 0.31–1.94) (Ruiz-Rodriguez 2014). Even taking into account the presence of this antinutrient with its ability of complexing mineral elements, these wild fruit species may be considered as an interesting contribution to the European diet. They often also have very low Na content (<20 mg/100 g) (see Table 8.15), such as in the case of *C. monogyna*, *A. unedo*, *Z. lotus*, and *Z. jujuba* (Tables 8.14 and 8.15).

Other bioactive compounds present in European wild fruits include fatty acids, tocopherols and phenolic compounds (see Table 8.16). The mature fruits of *M. communis* and *Prunus spinosa* have high MUFA proportion (oleic acid, 18:1, *n*-9), up to 72.1%, and *Z. lotus* and *R. ulmifolius* have high amounts of of PUFA with values up to 63.9%, while *A. unedo* has a high α-linoleic acid (18:3n3) content, up to 37.1% (Morales *et al*. 2013). Relating to α-tocopherol, *A. unedo* and *R. ulmifolius* are notable for containing values up to 8 mg/100 g fresh fruit.

As previously demonstrated by different authors, species such as *Arbutus unedo*, *Prunus spinosa*, and *Rosa canina* are good sources of bioactive compounds such as phenolic compounds, including anthocyanins (Tardío & Sánchez-Mata 2016). *Prunus spinosa* and *M. communis* fruits are very rich in phenolic compounds and particularly anthocyanins, with values up to 1964 mg/100 g in *P. spinosa*, which compared with other fruits can be considered as an extraordinary source of anthocyanins. Guimarães *et al*. (2013) reported the highest concentration of phenolic acids and flavone/ols in *P. spinosa* fruits, 3-*O*-caffeoylquinic acid and quercetin 3-*O*-rutinoside being the major compounds. (+)-Catechin was the most abundant compound in *A. unedo* and *R. canina* fruits. *Crataegus monogyna* fruits also presented very high phenolic levels, including a high flavonol content compared with other fruits studied (Ruiz-Rodríguez *et al*. 2014b). Rodrigues *et al*. (2012) identified cyanidin 3-*O*-glucoside, pelargonidin 3-*O*-glucoside and peonidin 3-*O*-glucoside in *C. monogyna* fruits, the major anthocyanin being cyanidin 3-*O*-glucoside, and also

quercetin 3-*O*-rutinoside and quercetin 3-*O*-glucoside as the major flavonols (Table 8.16).

8.6 Conclusion

Wild fruits are threatened by population pressure and human activities such as clearing of forested areas to set up farmlands. Fortunately, developing countries are endowed with many varieties of such indigenous food plants that have an outstanding potential to reduce nutritional deficiencies among vulnerable groups (children, pregnant women, etc.). The current status of underutilized fruit plants calls for an urgent research and development effort to promote conservation, bioprospection, and sustainable utilization (Tomar *et al*. 2015).

The consumption of autochthonous species available in the field which are adapted to soil and extreme climate contiditions offers the possibility to diversify the diet in order to provide the daily macro- and micronutrient requirements (protein, fiber, and carbohydrates) and also many bioactive compounds of great interest. Unfortunately, utilization of indigenous food plants has steadily declined mainly due to lack of knowledge about their nutrient value, resulting from the limited research available (Kiremire *et al*. 2002).

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9
Wild Plant-Based Functional Foods, Drugs, and Nutraceuticals

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9.1 Introduction

Wild plants were originally the main element in the human diet, culminating in the different cultures and societies of today. However, the establishment of agriculture led to the decline of consumption of wild plants in comparison to the cultivars that could be grown every year (Grivetti & Ogle 2000). Nevertheless, consumption of wild plants is still a tradition that remains in many cultures, either for their nutritional and health benefits or for sociocultural behaviors that characterize many societies (Groot *et al*. 2002; Pardo de Santayana *et al*. 2007; Schulp *et al*. 2014). As human health and nutrition are two of the pillars that sustain our survival, it is necessary to find new ways to support medical care, which can be found in the vast wild plant ecosystem (Heywood 2011).

Food with additional functional properties could be the future of health supplies for the world population, and thus food and drugs are increasingly seen as one matrix (Bernal *et al*. 2011). Functional foods, nutraceuticals, and drugs based on wild plants

that are still unexplored are emerging as a response to the world market, which has been searching for new, better, and safer products.

Functional foods have a similar appearance to their traditional counterparts, but bring potential beneficial effects when consumed on a regular basis in a varied diet. On the other hand, nutraceuticals are substances that have positive physiological effects on the human body, being consumed in unit dose forms such as tablets, capsules or liquids, allowing the delivery of a concentrated bioactive agent and providing a dose that could not be obtained from a normal food intake (Gulati & Ottaway 2006; Hasler 2000). Both the functional food and nutraceutical sectors have been growing significantly in Europe but in the European Union nutraceuticals are not considered as a specific food category with a series of rules and guidelines to define the product itself, obeying the general regulations on food safety, traceability, recall, and notification (Coppens *et al*. 2006; Gulati & Ottaway 2006). In terms of the health claims associated with functional foods, relating to Regulation (CE) No. 1924/2006 of the European Parliament on nutritional claims and health properties of food, it is possible to classify a functional food under very strict rules and conditions. In addition to the legislation required for all foodstuffs, scientific evidence of the health claims regarding the relevant food will be mandatory for all new products (Bech-Larsen & Scholderer 2007). In the United States, on the other hand, the Food and Drug Administration (FDA) defines the product's category depending on its characteristics, nutraceuticals being regulated as a food and beverage product and dietary supplement, covered by several safety issues, health claims, labeling, and good manufacturing practices (Milner 2000; Wrick 2005).

Concerning drug development, the market also requires safer products due to increasing worldwide concern about synthetic chemical compounds. In that respect, wild plant-based drugs are now in the forefront of the therapeutic agents used for human health, taking into account their high efficiency and low toxicity (Bhardwaj *et al*. 2014; Carocho & Ferreira 2013a).

In this chapter, wild plants commonly used as functional foods will be reviewed. For nutraceuticals, the emerging concept, their applications and novel formulations will be described, and also

some products already available on the market. The relationship between the bioactive phytochemical and the active principle will be explained, listing the common formulations in wild plant-based drugs and the different therapeutic targets that can be explored.

9.2 Wild Plants and Functional Foods

9.2.1 The Concept and Recent Trends in Functional Foods

In the first half of the twentieth century, the focus of nutritional science was on establishing the minimum requirements for essential nutrients that ensure the avoidance of deficiency diseases (MMWR 1999). Nowadays, these concepts are changing significantly in the industrialized world. We are progressing from a concept of "adequate nutrition" to one of "optimal nutrition" (Ashwell 2003); from a matter of survival, satisfying hunger, and ensuring food safety to an emphasis on the potential for foods to promote health, in terms of both preventing nutrition-related diseases and improving physical and mental wellbeing (Nöthlings *et al.* 2007; Takachi *et al.* 2008). In addition, consumers are increasingly better informed about the subject than they were in the past. As a result, their expectations of obtaining health benefits from the food they eat are also increasing (Diplock *et al.* 1999). These changes can be explained by some significant trends in our present society, namely rapid advances in science and technology, the rising costs of healthcare, the increase in the numbers of elderly people and in average life expectancy, changes in food laws affecting label and product claims, and people's desire for a better quality of life (Roberfroid 2007).

The primary role of food is to provide nutrients to meet human metabolic requirements and to give the consumer a feeling of satisfaction and wellbeing through hedonistic attributes such as taste. In addition to this, food can fulfill specific physiological functions in the human body (Li *et al.* 2014a; Zhang *et al.* 2015). In fact, food can not only help to achieve optimal health and

development, but it might also play an important role in reducing or preventing the risk of disease. According to the World Health Organization (WHO) and Food and Agriculture Organization (FAO), several dietary patterns along with lifestyle habits constitute major modifiable risk factors in relation to the development of coronary heart disease, different types of cancer, diabetes, obesity, osteoporosis, and periodontal disease (WHO 2003). Foods with these properties were first regulated in Japan in 1981 as Foods for Specified Health Use (FOSHU) (Hasler 2002; Ohama *et al.* 2006). Later, in Europe, the project Functional Food Science in Europe (FUFOSE) was created to assess critically the science base required to provide evidence that specific nutrients and food components beneficially affect target functions in the human body (Tijhuis *et al.* 2012). Currently, this kind of food is generally referred to as "functional food," if in accordance with the definition given below.

Although there is no universally accepted definition for functional foods (Hasler 2002), and because functional foods are more of a concept than a well-defined group of food products, here we present the definition described previously by Diplock *et al.* (1999). According to these authors, a food can be regarded as "functional" if it is satisfactorily demonstrated to affect beneficially one or more target functions in the human body, beyond adequate nutritional effects, in a way relevant to either an improved state of health and wellbeing and/or disease risk reduction. These foods must remain foods in appearance and they must demonstrate their effects in amounts that can normally be expected to be consumed in the usual diet, i.e. they are not pills or capsules, but part of a normal food pattern. Additionally, a functional food can be a natural or unmodified food, or one to which a component has been added or removed by technological or biotechnological means. It can also be a food where the nature of one or more components has been modified, the bioavailability altered, or any combination of these possibilities. Additionally, a functional food might be functional for all members of a population or for particular groups only. It is also important to note that, along with the nonuniversal definition, global markets also do not have the same regulatory systems for these foods (Bagchi 2014).

Functional food science is still at an early stage in its development. However, since knowledge about the functional effects of foods is increasing and the functionality of particular foods and food components is more extensively recognized, technology will have a continuing role to play in making those foods and food components more widely available and accessible (Howlett 2008). On the other hand, it is now known that genetic factors influence the relationship between diet and disease, and the ways in which different protective and risk factors can act. Furthermore, it is possible to visualize differences between genetic profiles of individuals at the molecular level and understand how they relate to differences between those individuals' responses to physiological factors. Thus, in the near future, knowledge gained in the fields of genomics, proteomics, and metabolomics (collectively known as "omics") will be of great importance for the development of functional foods and to create customized diet programs, as well as verifying the influence of dietary factors on human health and disease, which can lead to the identification of new food functionality routes (Howlett 2008).

9.2.2 Classification and Development of Functional Foods

Functional foods represent one of the most interesting and active areas of research and innovation in the food industry (Annunziata & Vecchio 2011). Their design and development, besides being an expensive process (Betoret *et al*. 2011), is a key issue, as well as a scientific challenge, which should rely on basic scientific knowledge relevant to target functions and their possible modulation by food components (Diplock *et al*. 1999). It is possible to separate them into natural (or nonaltered) and modified functional foods. But whether modified or not, they should always be safe, without any consideration of a trade-off between health benefit and health risk. More specifically, and according to the definition of functional foods presented before, they can be classified as:

- *nonaltered products*: foods naturally containing increased content of nutrients and/or health-promoting compounds
- *fortified products*: foods wherein the content of the existing components is increased
- *enriched products*: foods to which a component not normally found is added to provide benefits
- *altered products*: foods in which a component is removed or replaced by an alternative component with favorable properties
- *enhanced commodities*: the food composition is altered by changing the raw commodity, i.e. one of the components is enhanced through special growing conditions, breeding, or biotechnological means.

Although the functional food industry is growing steadily worldwide, the successful commercialization of new functional foods remains a challenge, especially due to the need for a strategic approach to their production processes (Howlett 2008). For this reason, during the development or reengineering of modified functional foods, it is necessary to take into account many variables, such as sensory acceptance, convenience, stability, chemical and functional properties, and price (Betoret *et al*. 2011; Granato *et al*. 2010). In fact, the relationship "structure-property" needs to be noted, once the functional effect depends on the active component gaining access to the functional target site. However, foods are mostly complex mixtures that can trap active compounds, modulate their release, or inhibit their activity. Thus, the food matrix in its raw state, after culinary preparation, or storage can have a significant influence on the activity or release of the key components. According to Betoret *et al*. (2011), the design of appropriate food vehicles to maintain the active form until the time of consumption, and to deliver this form to the desired target site within the organism, is vital to the success of functional foods.

Betoret *et al*. (2011) grouped the available technologies for functional foods development into three main categories. The first group is formed by the most commonly used technologies for functional foods development, including technologies traditionally used in food processing, formulation, and blending as well as for cultivation and breeding. The second group, constituted by

methodologies that form a structure to try to prevent the deterioration of physiologically active compounds, includes microencapsulation, edible films and coatings, and vacuum impregnation technologies. The third group, formed by recent technologies that are intended to design functional foods aimed at personalized nutrition, is the one that has grown significantly in recent years.

9.2.3 Wild Plants Used as Functional Foods

Plants are irreplaceable food resources for humans. Their interchangeable use as foods and as medicines, or healthy foods, has been part of human heritage since prehistoric times. Despite only a small number of existing plant foods having substantial clinical documentation of their health benefits, an even smaller number (and including only cultivated plants) have surpassed the rigorous standard of "significant scientific agreement" required by the FDA and EFSA for authorization of a health claim (Hasler 2002). Oat soluble fiber, soluble fiber from psyllium seed husk, soy protein and sterol and stanol ester-fortified margarine are plant-based foods currently eligible to bear an FDA-approved health claim (Hasler 2002). However, there is growing clinical research supporting the potential health benefits of various plant foods (including wild plants) or food constituents that currently do not have approved health claims, and thus are described as having "moderately strong evidence." Examples include berries, leafy vegetables, garlic, grapes and chocolate, among others listed in Table 9.1.

Table 9.1 Some wild edible plant foods claimed to have functional properties.

Plant species	**Common name and used part**	**Evaluated extract**	**Functional compounds**	**Potential health benefits**	**Reference**
Allium ampeloprasum	Wild leek (bulb)	Aqueous and	Fiber, zinc,	Antioxidant, antiinflammatory,	Adão *et al.* 2011;

L.		ethanolic extracts	polyunsaturated fatty acids (mainly palmitic acid), polysaccharides (glucofructan), and steroidal saponins	antiulcerogenic and gastroprotective	García-Herrera *et al.* 2014; Malafaia *et al.* 2015
Arbutus unedo L.	Strawberry tree (fruit)	Methanolic, hydromethanolic (80%), and phenolic extracts	Tocopherols, ascorbic acid, flavan-3-ols, and galloyl derivatives	Antioxidant activity and antitumor potential against NCI-H460 human cell line	Barros *et al.* 2010; Guimarães *et al.* 2013, 2014
Asparagus acutifolius L.	Wild asparagus (shoots)		Tocopherols, vitamin C, and glycosides of flavonols	Antioxidant	Barros *et al.* 2011b; Martins *et al.* 2011
Beta spp.	Beet (root)	Aqueous, hydroethanolic, methanolic, and betalain-rich extracts and juice	Phenolic acids (ferulic, vanillic, *p*-hydroxybenzoic, caffeic and protocatechuic acids),	Antioxidant, hepatoprotective, anticancer, antiproliferative activity in MRC5 and MCF-7 cell lines, antihypertensive	Wootton-Beard & Ryan 2011; Ninfali & Angelino 2013; Vulić *et al.* 2014

			flavonoids (catechin, epicatechin, rutin and vitexin), betalains (betanin, isobetanin and vulgaxanthin I), minerals (potassium, magnesium, iron, zinc, calcium, sodium), folic acid, biotin and soluble fiber	and hypoglycemic	
Capparis decidua (Forssk.) Edgew.	Caper or kair (fruit)	Aqueous, methanolic, acidified methanolic, hydroalcoholic and ethanolic extracts	N-pentacosane, β-sitosterol, β-carotene, alkaloids, phenolic compounds including flavonoids and minerals (manganese, copper, and iron)	Antioxidant, antidiabetic, diuretic, hypercholesterolemic, antihypertensive, antiatherosclerotic, hypolipidemic, antimicrobial, and anthelmintic	Rathee *et al.* 2010 ; Sharma *et al.* 2010, Zia-Ul-Haq *et al.* 2011 ; Saad *et al.* 2014

Dimocarpus longan Lour.	Longan (fruit)	Aqueous, hydromethanolic (80%), acetone:ethanol (1:1, v/v), hydroacetonic (70%) and polyphenol-rich extracts	Phenolic compounds (corilagin, gallic and ellagic acids, flavone glycosides, glycosides of quercetin and kaempferol and epicatechin), vitamin C, fiber, and minerals	Antioxidant, antiinflammatory, antityrosinase, antiglycated, anticancer, and memory-enhancing effects	Huang *et al*. 2010a; Yang *et al*. 2011; Huang *et al*. 2012
Eugenia uniflora L.	Pitanga or Brazilian cherry (fruit)	Ethyl acetate and ethanolic extracts	Anthocyanins, flavonols, and carotenoids	Antidiarrheic, diuretic, antirheumatic, antifebrile, antidiabetic, antimicrobial, and antitrypanosoma	Costa *et al*. 2013
Euterpe oleracea Mart.	Acai, assai or açaí (fruit)	Ethyl acetate, *n*-buthanolic, hydromethanolic (50%), and hydroacetonic (70%) extracts	Flavonoids (anthocyanins and proanthocyanidins), phenolic acids, lignans, and stilbenes	Antioxidant, antiallergic, anticancer, antiinflammatory, atheroprotective, improves the endothelial function and	Rufino *et al*. 2010 ; Kang *et al*. 2011; Costa *et al*. 2013

				platelet aggregation, vasodilation, and prevents cardiovascular disease	
Ficus carica L.	Fig (fruit)	Hexane, methanolic, and hydromethanolic extracts	Phenolic acids (chlorogenic acid), anthocyanins, flavonols, flavones (luteolin), minerals (iron, potassium, sodium and calcium), fiber, sugars, and vitamin A	Antioxidant, anticholinesterase, anticarcinogenic, antiproliferative activity in several cancer cell lines, digestive, antifungal, and anthelminthic	Huang *et al*. 2010a; Barolo *et al*. 2014; Shad *et al*. 2014
Fragaria vesca L.	Wild strawberry or European strawberry (fruit)	Aqueous extracts and combined extract of *n*-buthanolic and to HCl (1 mol/dm^3)	Flavonoids (e.g. anthocyanins), phenolic acids, and salicylic acid	Antioxidant	Najda *et al*. 2014
Garcinia mangostana	Mangosteen or purple	Aqueous, methanolic,	Xanthones (α-, β-,	Antioxidant, antitumor,	Pedraza-Chaverri

L.	mangosteen (fruit)	ethanolic, hydroethanolic (40 and 50%), and juice extracts	and γ-mangostins, garcinone E, 8-deoxygartanin and gartanin)	antiproliferative, proapoptotic, antiinflammatory, antiallergic, antibacterial, antifungal, antiviral, antimalarial, antidiabetic, antihiperlipidemic and antiatherogenic, cardioprotective, hepatoprotective, immunomodulator, and antiulcer	*et al.* 2008; Gutierrez-Orozco & Failla 2013
Gardenia jasminoides J. Ellis	Gardenia (fruit)	Hydromethanolic (80%), hydroethanolic (60%), and cetone:ethanol (1:1, v/v) extracts	Caffeoylquinic acid derivatives (chlorogenic acid, dicaffeoylquinic acid and other caffeoyl-conjugate quinic acid derivatives), flavonoids (rutin), iridoids (geniposide), and carotenoids (crocin)	Antioxidant and antiinflammatory	Huang *et al.* 2010a; Peng *et al.* 2013

Litchi chinensis Sonn.	Litchi or lychee (fruit)	Methanolic (70% and 80%), acetone:ethanol (1:1) and juice extracts	Phenolic compounds (cinnamic acid and procyanidins), carotenoids, and vitamin C	Antioxidant, antiapoptotic, and hepatoprotective	Huang *et al.* 2010a; Bhoopat *et al.* 2011; Lv *et al.* 2014
Lycium barbarum L.	Goji berry (fruit)	Aqueous, methanolic, and crude and purified polysaccharide extracts	Polysaccharides, carotenoids (zeaxanthin), betaine, cerebroside, β-sitosterol, *p*-coumaric acid, and vitamin C	Antioxidant, antiaging, antiinflammatory, anticancer, cytoprotective, neuroprotective, metabolism stimulator, glucose regulator in diabetics, glaucoma (eye health benefits), immunomodulatory, antibacterial and cardioprotective	Amagase & Farnsworth 2011
Malpighia emarginata D.C.	Acerola or wild crepe myrtle (fruit)	Methanolic, hydromethanolic (50%), hydroacetonic (70%), and aqueous extracts and juice	Vitamin C, phenolic, carotenoids (β-carotene), riboflavin, thiamine, fiber, minerals	Antioxidant, antiaging, antiinflammatory and prevents weight gain and dyslipidemia	Mezadri *et al.* 2008; Rufino *et al.* 2010 ; Delva & Goodrich-Schneider 2013;

			(phosphorus, calcium, and iron), anthocyanins (cyanidin-3-rhamnoside and pelargonidin-3-rhamnoside) and flavonols (quercetrin)		Dias *et al*. 2014
Montia fontana L.	Water blinks (aerial parts)	Methanolic extract	Tocopherols and vitamin C	Antioxidant	Pereira *et al*. 2011; Morales *et al*. 2012
Myrciaria cauliflora (Mart.) O. Berg	Jaboticaba or guapuru (fruit)	Methanol:formic acid (9:1, v/v), methanol:water:acetic acid (85:15:0.5, v/v/v/), methanolic, hydromethanolic (50%), ethanolic, acetonic, and hydroacetonic (70%) extracts	Anthocyanins, ellagic and gallic acid, carotenoids, depsides, tannins, cutin, and vitamin C	Antioxidant, antiinflammatory, inhibits IL-8 production, antiproliferative effects against tumor cells, protective effect in cardiovascular disease and type 2 diabetes mellitus	Rufino *et al*. 2010; Leite *et al*. 2011; Costa *et al*. 2013
Myrciaria	Camu-	Hydromethanolic	Anthocyanins,	Antioxidant,	Rufino *et*

dubia (Kunth.) McVaugh	camu, cacari or camocamo (fruit)	(50%) and hydroacetonic (70%) extracts	myricetin and conjugates, ellagic acid and conjugates, ellagitannins, flavan-3-ols, proanthocyanidins, and vitamin C	antiinflammatory and inhibits the LPS-induced NO release in RAW 264.7 cells	*al.* 2010 ; Costa *et al.* 2013; Fracassetti *et al.* 2013
Nasturtium officinale W. T. Aiton	Watercress (aerial parts)	Methanolic and hydromethanolic (70%) extracts	Phenolic compounds and minerals (phosphorus, potassium, calcium, and manganese)	Antioxidant, anticarcinogenic, and chemopreventive	Pereira *et al.* 2011; Manchali *et al.* 2012
Origanum vulgare L.	Oregano (aerial parts)	Aqueous (infusions and decoctions) and hydromethanolic extracts (80%)	Flavonoids and phenolic acids	Antioxidant and antimicrobial potential	Martins *et al.* 2014
Physalis spp.	Physalis or golden berry (fruit)	Hydroethanolic (70%) extracts	Physalins, withanolides, sterols, polysaccharides, and flavones	Antiinflammatory, antioxidant, antitumor, hypoglycemic, and analgesic	Li *et al.*, 2014c
Prosopis cineraria	Ghaf, khejri,	Aqueous and	Triterpenoids (3-	Antioxidant and	Liu *et al.* 2012

(L.) Druce	sami or golden tree of Indian deserts (pod)	methanolic extracts	benzyl-2-hydroxy-urs-12-en-28-oic acid and maslinic acid-3 glucoside), fatty acid (linoleic acid), piperidine alkaloid (prosophylline) and polyphenols (5,5′-oxybis-1,3-benzenediol, 3,4,5-trihydroxycinnamic acid 2-hydroxyethyl ester and 5,3′,4′-trihydroxyflavanone 7-glycoside)	antiinflammatory	
Prunus spinosa L.	Blackthorn or sloe (fruit)	Methanolic and phenolic enriched extracts	Ascorbic acid, phenolic acids and flavonoids (anthocyanins, flavonols and	Antioxidant and antitumor potential	Barros *et al*. 2010; Guimarães *et al*. 2013, 2014

			flavones)		
Psidium cattleianum Sabine	Strawberry guava (fruit)	Hexane, ethyl acetate, acetonic, aqueous, ethanolic, and methanolic extracts	Phenolic compounds (ellagic acid, ellagic acid deoxyhexoside and epicatechin gallate), carotenoids, vitamin C, and fiber	Antioxidant, antiinflammatory and antimicrobial	McCook-Russell *et al*. 2012; Ribeiro *et al*. 2014
Psidium guajava L.	Guava (fruit)	Methanolic, hydromethanolic (80%), acetone:ethanol (1:1, v/v), hexane, ethyl acetate and ethanol/water/formic acid (70:25:5, v/v/v) extracts	Phenolic acids (chlorogenic acid), flavan-3-ols (catechin), anthocyanins (delphinidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside)	Antioxidant, antiinflammatory, and antimicrobial	Huang *et al*. 2010a; McCook-Russell *et al*. 2012; Flores *et al*. 2015
Punica granatum L.	Pomegranate (fruit)	Aqueous, ethyl acetate, acetonic, and methanolic	Anthocyanins, gallotannins, ellagitannins (ellagic acid, gallic	Antioxidant, antiinflammatory, antiallergic, chemopreventive, anticancer, cardioprotective,	Ismail *et al*. 2012

		extracts	acid, and punicalagin), gallagyl esters, hydroxybenzoic and hydroxycinnamic acids and dihydroflavonol	gastroprotective, antimicrobial, and anthelminthic	
Rhodomyrtus tomentosa (Aiton) Hassk.	Rose myrtle (fruit)	Hexane, methanolic, hydromethanolic (80%), acetone:ethanol (1:1, v/v), acetone:water:acetic acid (50:49:1, v/v/v), and flavonoid-rich extracts	Flavonoids (gallocatechin gallate, dihydromyricetin, quercetin, kaempferol, anthocyanins and vitexin), organic acids, polysaccharides, fiber, vitamin E (α-tocopherol), minerals (manganese and copper) and essential fatty acids (mainly linoleic acid)	Antioxidant	Huang *et al*. 2010a; Lai *et al*. 2015; Wu *et al*. 2015
Rubus	Blackberry	Hexane,	Anthocyanins,	Antioxidant,	Bowen-

spp.	and raspberry (fruits)	ethyl acetate, and methanolic extracts	flavonols, phenolic acids (ellagic acid), vitamins C and E, folic acid, and β-sitosterol	antiinflammatory, and chemopreventive	Forbes, *et al.* 2010
Sambucus nigra L.	Elderberry, black elder or European elder (fruit)	Methanolic, acidified methanolic, ethanolic, and hydroethanolic (80%) extracts	Polyphenols (anthocyanins, flavonols, phenolic acids and proanthocyanidins), terpenes, lectins, unsaturated fatty acids, fiber, vitamins A, B, C and E, and minerals	Antioxidant, cardiovascular protection, antidiabetic and antiobesity, reinforces the immune system, antiviral, antibacterial, and UV radiation protector	Barros *et al.* 2011a; Sidor & Gramza-Michałowska 2014
Syzygium cumini (L.) Skeels.	Jambul or jambolan (fruit)	Methanolic, hydromethanolic (50%), hydroacetonic (70%), and hexane extracts	Anthocyanins, phenolic acids (ellagic acid), flavonols (quercetin and rutin), carotenoids,	Antioxidant, antiscorbutic, diuretic, and antidiabetic	Rufino *et al.* 2010; Costa *et al.* 2013; Shad *et al.* 2014

			vitamin C, and manganese		
Vaccinium myrtillus L.	Bilberry (fruit)	Acidified methanolic, ethyl acetate, hexane, anthocyanins, and proanthocyanidin-rich extracts	Flavonoids (proanthocyanidins and anthocyanins), carotenoids (lutein and zeaxanthin), and sterols	Antioxidant, antibacterial (inhibition of urinary tract infections), anticarcinogenic and antiproliferative activity in two human breast cancer cell lines MCF-7 and BT-20	Madhavi *et al.* 1998
Vaccinium spp.	Cranberry (fruit)	Hydroacetone (80%), ethyl acetate, and phenolic extracts	Phenolic acids and flavonoids (anthocyanins, proanthocyanidins, and flavonols)	Antioxidant, antiinflammatory and cardiovascular, and urinary tract protection	Singh *et al.* 2009; Khoo & Falk 2014
Zingiber officinale Rosoe	Ginger (rhizome)	Aqueous and methanolic extracts	Gingerols (6-gingerol), shogaols (6-shogaol), fiber, and flavonoids	Antioxidant, antiinflammatory, antithrombotic, and cholesterol lowering, analgesic, antipyretic,	Thomson *et al.* 2002; Mojani *et al.* 2014

				and hypotensive	
Ziziphus jujuba Mill.	Jujube or red date (fruit)	Aqueous, hexane, methanolic, and hydromethanolic extracts	Saponins, tannins, terpenoids, flavonoids, and iron	Antioxidant, antiinflammatory, and gastrointestinal protector	Yu *et al.* 2012; Shad *et al.* 2014

Table 9.1 presents wild edible plants that have been investigated due to their claimed functional properties. These plants are interesting sources of physiologically active ingredients which are linked to various beneficial health effects. Various berries, including elderberry, bilberry, cranberry, blackberry, raspberry, and wild strawberry, stand out as a source of anthocyanins, proanthocyanidins, flavonols, phenolic acids, and vitamins, among other bioactive compounds. These molecules, isolated or in combined extracts, have antioxidant, antiinflammatory, anticarcinogenic, cardioprotective, and antibacterial properties (Barros *et al*. 2011a; Bowen-Forbes *et al*. 2010; Madhavi *et al*. 1998; Najda *et al*. 2014; Sidor & Gramza-Michalowska 2014; Singh *et al*. 2009). Wild strawberry fruits harvested from natural habitats were highlighted by Najda *et al*. (2014) as containing more anthocyanins and higher antioxidant activity than those from cultivation. Likewise, Lv *et al*. (2014) showed that the wild litchi cultivar Hemaoli has high total phenolic and flavonoid content in comparison to one of the main market cultivars. This fruit also has high levels of carotenoids and vitamin C, which contribute to its antioxidant, antiapoptotic, and hepatoprotective effects (Bhoopat *et al*. 2011; Huang *et al*. 2010a; Lv *et al*. 2014). Physalis (*Physalis* spp.) is another berry with claimed functional properties. Physalins, withanolides, sterols, polysaccharides, and flavones are compounds present in this golden berry. According to Li *et al*. (2014c), it has antiinflammatory, antioxidant, antitumor, hypoglycemic, and analgesic properties.

Other plants, like the root of beet (*Beta* spp.), have antioxidant, hepatoprotective, anticancer, and antiproliferative activity in MRC5 and MCF-7 cell lines, antihypertensive, and hypoglycemic

effects. These health benefits are conferred by the high content of phenolic acids, flavonoids, betalains, minerals (P, Mg, Fe, Zn, Ca, and Na), folic acid, biotin, and soluble fiber (Ninfali & Angelino 2013; Vulić *et al*. 2014; Wootton-Beard & Ryan 2011). In turn, ginger (*Zingiber officinale* Rosoe) has been described as a source of gingerols (6-gingerol), shogaols (6-shogaol), fiber, and flavonoids, as well as having antioxidant, antiinflammatory, antithrombotic, cholesterol-lowering, analgesic, antipyretic, and hypotensive effects (Mojani *et al*. 2014; Thomson *et al*. 2002).

Regarding leafy vegetables, the aerial parts of water blinks (*Montia fontana* L.) have high amounts of tocopherols and vitamin C, compounds that provide antioxidant benefits (Morales *et al*. 2012; Pereira *et al*. 2011), while watercress (*Nasturtium officinale* W.T. Aiton) is a rich source of phenolic compounds and minerals (P, Mg, Ca, and Mn) which confer its claimed antioxidant, anticarcinogenic, and chemopreventive effects (Manchali *et al*. 2012; Pereira *et al*. 2011). The aerial parts of oregano (*Origanum vulgare* L.), prepared in infusions, decoctions or hydromethanolic extracts (80%), have antioxidant and antimicrobial potential probably related to flavonoids and phenolic acids (Martins *et al*. 2014).

Today, aggressive marketing highlighting the health-promoting benefits of mangosteen, acai, acerola or goji berry, among other fruits, bulbs, roots, seeds or leafy vegetables presented in Table 9.1, has resulted in their classification as "superfruits" or "superfoods." Scientific research carried out in recent years proves their effectiveness as healthy foods, and due to high profits, the food and pharmaceutical industries are increasingly interested in developing new products based on these plants.

However, in addition to edible plant parts, wild nonedible parts or plants can also be used as a source of health-promoting ingredients. Thus, medicinal and aromatic plants play an important role in the development of new or improved functional foods, as well as nutraceuticals. At the research level, some wild plant extracts are being incorporated into food products to increase their health-promoting properties. Martins *et al*. (2014) formulated new yogurts based on phenolic extracts of wild blackberry (*Rubus ulmifolius* Schott) flowers. The authors microencapsulated the

hydroalcoholic extract in an alginate-based matrix and incorporated this into a yogurt to achieve antioxidant benefits. Recently, Caleja *et al*. (2015) improved the antioxidant properties of cottage cheese by the incorporation of fennel (*Foeniculum vulgare* Mill.) decoction (phenolic-enriched extract), improving not only functionality of the final product but also preservation effectiveness due to the antimicrobial potential of fennel.

Carocho *et al*. (2015a) transformed the Portuguese "Serra da Estrela" cheese into a functional food by incorporating dried chestnut (*Castanea sativa* Mill.) flowers or lemon balm (*Melissa officinalis* L.) plants, as well as their decocted extracts. The functionalized cheeses showed higher antioxidant activity, especially lipid peroxidation inhibition, bringing benefits both for consumers (healthier product) and producers (added-value product). The same authors also functionalized the Portuguese traditional cakes "económicos" by incorporation of dried chestnut (*C. sativa*) flowers or decoctions prepared from them (Carocho *et al*. 2015b). The final product showed increased antioxidant activity and phenolic content, without causing visible changes in inner and outer appearance.

9.3 Wild Plant-Based Nutraceuticals

9.3.1 The Emerging Concept and Applications of Nutraceuticals

A new generation of processed food is coming, which is a controversial subject for many people. Nutraceutical products represent a fast-growing sector within the food industry, aiming to increasingly attract the buyer to consume these novel dietary supplements and phytotherapeutic products. It is expected that in the near future, "food for special dietary needs," such as soups, smoothies, processed meat, bread and sausages, among others, will be enriched with nutraceutical formulations (Andlauer & Furst 2002; Regulation (EC) No. 2002/46).

Nutraceuticals can be defined as diet supplements that contain bioactive compounds or extracts, prepared from raw natural matrices that will provide a higher dosage that could not be obtained from normal food products and functional foods (DeFelice 1992; Espín *et al*., 2007; Zeisel 1999). Directive 2002/46/EC of the European Parliament and Council, on the approximation of laws of Member States relating to food supplements, defines "food supplements" as foodstuffs with the purpose of supplementing the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, such as capsules, pastilles, tablets, pills, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities (Regulation (EC) No. 2002/46).

The health industry is using nutraceutical formulations as complements to prevent some diseases. Some authors have stated that any food or parts of foods can be considered nutraceutical compounds, as long as their beneficial health and nutritional claims are proved scientifically (Braithwaite *et al*., 2014; McNamara, 1997; Ross, 2000). On the other hand, Gulati and Ottaway (2006) and Espín *et al*. (2007) distinguished nutraceuticals as components that are often consumed in unit dose forms such as tablets, capsules or liquids. They can be isolated nutrients or herbal products presented in pharmaceutical forms or processed products like cereals, smoothies, and soups for special diet requirements (Andlauer & Furst 2002; Braithwaite *et al*. 2014; Regulation (EC) No. 2002/46).

The concept of nutraceuticals is relatively recent, only appearing in the 1990s with the first publications and patents related to the subject (Figure 9.1). However, the increasing number of publications from academics (through articles and reviews) and industry (through patents) is notable. This can be explained by the fact that there is increasingly market demand for new, better, and safer food products. However, regarding plant-based nutraceuticals, the number of articles (and reviews) and patents is very low (see Figure 9.1) although it is growing. Many of the primary studies on nutraceuticals were made with individual

compounds with known beneficial effects, but there is now interest in exploring the synergisms existing within plant extracts and incorporating them into nutraceuticals or modified functional food.

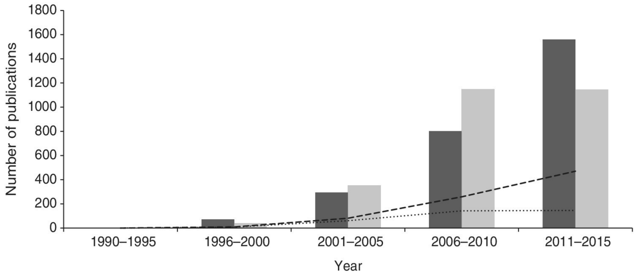

Figure 9.1 Number of research articles and reviews (■ and - - -), and patents (■ and.........) published in the period from 1990 to 2015 regarding nutraceuticals and nutraceuticals formulated with plant material, respectively (obtained on Web of Science, January 2015; keyword: nutraceutical; nutraceutical + plant).

Therefore, the development of legislation that regulates the production of nutraceutical formulations, their labeling and market supply is crucial. In January 2002, the European Food Safety Authority (EFSA) established Regulation (EC) No. 178/2002, setting down the general principles and requirements for food law. This Regulation also contains procedures for food safety, increasing the health protection of consumers. These guidelines are applied to all food products, including those with added functional properties, such as nutraceuticals and functional foods. From the perspective of the global pharmaceutical and medical industry, nutraceutical products are dietary supplements (Kwak & Jukes 2001). Specific regulation of nutraceutical products is still very patchy; in European law they have no specific category, being considered under the same parameters used for dietetic foods, dietetic supplements, and food supplements (Coppens *et al*. 2006; Regulation (EC) No. 2002/46) or even under medicinal classification (Gulati & Ottaway 2006). In the USA, nutraceuticals are considered only as dietary supplements (Bernal *et al*. 2011; Espín *et al*. 2007). The differences between European and USA regulation may be due to cultural, historical, and traditional backgrounds (Gulati & Ottaway 2006). However, the development

of specific legislation in Europe is necessary to ensure food safety for consumers and to prove that nutraceuticals are safe and scientifically accepted, and this may dictate the future success of these products (Braithwaite *et al.* 2014; Byrne 2003).

Recent research has shown very promising prospects for different natural ingredients added to food products, creating benefits for consumers' health and added value for manufacturers (Coppens *et al.* 2006). Many of the published papers on nutraceuticals are focused on their beneficial health properties (Bernal *et al.* 2011), for instance their ability to decrease the development of heart disease (Garcia-Rios *et al.* 2013; Giordano *et al.* 2012; Izzo *et al.* 2010; Scicchitano *et al.* 2014) such as hypercholesterolemia (Mannarino *et al.* 2014), and also for the prevention and treatment of prostate cancer (Li *et al.* 2014b). Nutraceutical formulations have been proved to be safe and well tolerated, but further studies are required to assess the decreasing of secondary effects of nutraceuticals when compared to analogue commercial drugs for the treatment of certain diseases (Bernal *et al.* 2011; McAlindon 2006).

9.3.2 Recent Advances in Formulations for Nutraceuticals

Due to the difficulty in the classification of nutraceuticals, we are faced with two types of products: nutraceuticals in the form of dietary supplements (tablets, capsules, solutions, syrups, powders, chewing tablets, among others) and those in the form of free or encapsulated extracts/compounds to be inserted into a food matrix (i.e. used to develop functional foods). For that reason, the formulation of nutraceuticals involves a wide range of methodologies and techniques, from the most used (tableting) to the newest and most advanced, such as microencapsulation complemented with nanotechnology.

First, it is necessary to ensure the safety and quality of the nutraceutical product. The chemical, nutritional, and bioactive characterization of the compound/extract that will be part of the formulation is required, as well as control of the dosage. For this,

some advanced analytical techniques are used such as mass spectrometry (MS), nuclear magnetic resonance (NMR), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), and gas chromatography (GC), among others (Bernal *et al*. 2011; Sener & Orhan 2005).

The vast majority of nutraceutical formulations are designed for oral administration. Braithwaite *et al*. (2014) reported a description of some new nutraceutical formulation strategies to improve dosage, design, and delivery of the bioactives. From liposomal carriers, electrospun fiber mats, microsponges and nanoesponges, cyclodextrin complexations to biodegradable hydrogels, all these technologies prove the importance of nutraceuticals in today's economy, with a growing investment by the industry in new formulations that respond to market demand. Second-generation nanocrystals, another new formulation, are an emerging technology for the delivery of poorly soluble bioactives. They are mostly used for drug delivery to solve poor solubility and bioavailability. However, they also represent a reliable response for the delivery of many nutraceutical compounds already on the market, such as antioxidants. The main advantage of nanocrystal systems is the capacity to be applied via oral, intravenous, dermal, mucosal, ocular and even pulmonary routes (Shegokar & Müller 2010).

It is important to realize that nutraceutical formulations go far beyond diet products or products enriched with a certain bioactive compound. Formulations are already on the "micro" and "nano" scales, which can be incorporated in food matrices but also in pharmaceutical formulations, serving as a complement to traditional medicine. Microencapsulation complemented with nanotechnology appears to overcome problems related to the use of free bioactives but also to provide controlled target delivery release (Braithwaite *et al*. 2014; Dias *et al*. 2015 Ezhilarisi *et al*. 2013; Huang *et al*. 2010b). Nanoscale delivery systems have the advantages of improving solubility, masking undesirable flavors and smells, and preventing the degradation of the bioactive compounds; they provide a triggered controlled release and, most important of all, increased bioavailability by prolonging contact within the gastrointestinal tract (Cerqueira *et al*. 2014). Microemulsions, for instance, are one of the most used techniques

for the solubilization and transport of water and oil-insoluble compounds, presenting easier formulation and manufacture and also high stability during storage (Spernath & Aserin 2006).

Food protein-based materials can also be used at “micro” and “nano” scales, depending on the type of encapsulation methodology used to produce the capsules and also the objective of the work. Proteins present the ability to form gels and emulsions due to their functional properties, which makes them appealing to the industry and academia for the encapsulation of nutraceuticals (Chen *et al*. 2006). Hydrocolloids fibers are being proposed to encapsulate nutraceutical compounds and extracts; they are nontoxic, inexpensive, and generally recognized as safe (GRAS). Furthermore, since they are complex carbohydrates, consumption on a regular basis showed health benefits for cardiovascular disease and diabetes (Janaswamy & Youngren 2012). Researchers are also developing formulations linking nutraceuticals with drugs to enhance efficacy and reduce dosage and side-effects of chemical compounds (Braithwaite *et al*. 2014).

9.3.3 Examples of Nutraceuticals Based on Wild Plants

For economic and ecological sustainable reasons, the FAO recommends the cultivation of medicinal and aromatic plants that represent a genetic pool of raw material with better control of biotic and abiotic factors, allowing the standardization of the final product (Schippmann *et al*. 2002). For that reason all the listed examples in Table 9.2 are plants that are normally consumed as wild and that present some bioactive properties, allowing the development of nutraceutical formulations. In this chapter, we only discuss nutraceutical formulations in the form of dietetic supplements (capsules, tablets, syrup). A detailed description of microencapsulated nutraceuticals based on plants has been previously provided by Dias *et al*. (2015), where the most frequently used microencapsulation techniques and materials are described, and also the most common extracts and bioactive compounds, including also some applicability studies for the developed microcapsules (e.g. milk, cheese, yogurt, ice cream,

pasta, meat, bread, and chewing gum enhanced with bioactive extracts of plant origin).

Table 9.2 Nutraceutical formulations based on plants with traditional wild use.

Plant species	**Used part**	**Extract**	**Formulation**	**Application**	**Available commercial product**	**Reference**
Abelmoschus manihot L.	–	Standardized extract	Pills	Renal inflammation, glomerular; chronic kidney disease	–	Tu *et al*. 2013
Artemisia annua L.	Leaves	Dried leaves	Tablet	Antimalarial drug	–	Weathers & Towler 2014
Berberis aristata D.C. *Silybum marianum* (L.) Gaertn.	Root Fruit	Standardized extract	Tablet	Glycemia and lipid alteration	Berberol®	Pierro *et al*. 2013
Boswellia serrate Triana & Planch.	–	Gum extract	Pills	Osteoarthritis	5-Loxin®	Sengupta *et al*. 2010
	–	Gum extract + essential oil	Pills	Osteoarthritis	Aflapin®	Sengupta *et al*. 2010
Cajanus cajan L.	Seed		Syrup	Sickle cell	Ciklavit®	Imaga *et al*. 2013

				anemia		
Commiphora angolensis Welw.	Roots	Aqueous extract	Syrup, Pills	Antihepatocellular carcinoma	–	Pereira *et al.* 2013b, 2014
Cucumis melo cantalupensis	Fruit	Juice	Pills	Stress and fatigue	Extramel®	Milesi *et al.* 2009
Cynara scolymus L.	Leaves	Aqueous extract	Syrup, Pills	Antihepatocellular carcinoma	–	Pereira *et al.* 2013b, 2014
Echinacea angustifolia (D.C.) Hell.	Root	Polysaccharide extract	Syrup	Immunomodulatory effect	Polinacea®	Dapas *et al.* 2014
Echinacea purpurea (L.) Moench	Leaves and root	Dried leaves and roots	Pills	Cold	Echinaforce®	Brinkeborn *et al.* 1989
Echinacea purpurea (L.) Moench *Echinacea angustifolia* (D.C.) Hell.	Root	Ethanolic extract	Tablet	Bioavailability of bioactive compounds	–	Matthias *et al.* 2007
Echinacea purpurea (L.) Moench *Glycyrrhiza glabra* L.	Root	Standardized extract	Tablet	Immunological effects	Revitonil®	Wagner & Jurcic 2002
Ficus carica	Fruits	–	Syrup	Bioactive compounds	–	Puoci *et al.* 2011

L.						
Gingko biloba L.	Leaves	Aqueous extract	Syrup, Pills	Antioxidant activity	–	Pereira *et al*. 2013a
	Leaves	–	Tablet	Mild cognitive impairment	–	Bäurle *et al*. 2009
Gingko biloba L. *Panax ginseng* sp.	Leaves Root	Standardized extract	Pills	Mild cognitive impairment	Memo®	Yakoot *et al*. 2013
Ginseng panax sp.	Root	Standardized extract	Tablet	Antiaging	Eufortyn®	Xu *et al*. 2010
Hedera helix Linné L.	Leaves	Dry leaves	Tablet	Cough	Prospan®	Stauss-Grabo *et al*. 2011
Hypericum perforatum L.	Shoot tips	–	Tablet	Depression	–	Lenoir *et al*. 1999
Juglans regia L.	Leaves	Ethanolic extract	Pills	Hyperglycemia	–	Hosseini *et al*. 2014
Magnolia officinalis Rehder & Wilson *Phellodendron amurense* Rupr.	Bark	Standardized extract	Pills	Reducing stress and anxiety	Relora®	Talbott *et al*. 2013
Mikania laevigata Willd.	–	–	Syrup	Antispasmodic and respiratory diseases	–	Graça *et al*. 2007

Murraya koenigii (L.) Sprengel *Tribulus terrestris* L.	Leaves	Standardized extract	Pills	Benign protastic hyperplasia	–	Sengupta *et al.* 2011
Peumus boldus L.	Leaves	Dried leaves	Tablet	Choleretic, diuretic, stomachic, cholagogic properties	–	Palma *et al.* 2002
Phellodendron amurense Rupr. *Citrus sinensis* (L.) Osbeck	Bark Peel	Standardized extract–	Pills–	Joint pain and movements–	—	Oben *et al.* 2009–
Phoenix canariensis Chabaud	Sap	–	Syrup	Sugar and nutritional source	–	Luis *et al.* 2012
Phyllanthus amarus Schum. & Thonn	Leaves and stems	–	Syrup	Antitussive	–	Avbunudiogba *et al.* 2013
Proposis pods L.	Fruit	–	Syrup	Bioactive compounds	–	Quispe *et al.* 2014
Salvia officinalis L.	Leaves	Ethanolic water extract	Pills	Glycemia	–	Kianbakht & Dabaghian 2013
Silybum marianum	Bark	Aqueous extract	Syrup, Pills	Antihepatocellular carcinoma	–	Pereira *et al.*

(L.) Gaertn.						2013b, 2014
Smallanthus sonchifolius Poepp. & Endl	Root	–	Syrup	Obesity and insulin resistance	–	Genta *et al*. 2009
Uncaria tomentosa (Willd.) D.C.	Bark	Aqueous extract	Pills	Macrophage resistance	–	Lenzi *et al*. 2013
Vitis vinifera L. cv. *Cabernet sauvignon; Vitis vinifera* L. cv. *Merlot*	Grape	–	Tablet	Bioavailability of resveratrol	–	Ortuño *et al*. 2010

Currently, some trademarked plant-based nutraceuticals are used as adjuvants in several illness processes. For instance, Ciklavit® is a syrup which has effects again sickle cell anemia, prepared with an aqueous extract of *Cajanus cajan* L. seed, a plant found in semiarid tropical regions, commonly consumed in soups and rice dishes (Imaga *et al*. 2013). Xu *et al*. (2010) studied the effect of a commercial product, Eufortyn®, that comprises chemical compounds including terclatrated coenzyme Q_{10} and creatine, but also an extract of *Ginseng panax* sp. roots, which has shown effects in the antiaging process of rats. The roots of *Echinacea purpurea* (L.) Moench and *Glycyrrhiza glabra* L. are the major constituents of a commercial tablet, Revitonil®, used mainly for its immunological effects, while the leaves of *Hedera helix* L. are used in Prospon®, marketed for cough symptoms (Stauss-Grabo *et al*. 2011; Wagner & Jurcic 2002). Extramel® consists of small pills of melon juice (*Cucumis melo* var. *cantalupensis* Naudin) used to treat stress and fatigue symptoms (Milesi *et al*. 2009), while 5-Loxin® and Aflapin® contain gum extract and gum

extract plus oil, respectively, of *Boswella serrata* Triana & Planch, being used for osteoarthritis (Sengupta *et al*. 2010).

The genus *Echinacea* is well known for its medicinal properties. Brinkeborn *et al*. (1989) reported the effects of pills (Echinaforce®) prepared from *E. purpurea* roots and leaves in the treatment of the common cold, while Dapas *et al*. (2014) studied the root syrup (Polinacea®) obtained using *E. angustifolia* (D.C.) Hell. for its immunomodelatory effects. Berbenol®, a tablet formulation made from an extract of *Berberis aristata* D.C. and *Silybum marianum* (L.) Gaertn., taking advantage of the synergistic effects of both plants, is used for the treatment of glycemia and lipid value alterations in patients with type 2 diabetes (Pierro *et al*. 2013).

However, most of the formulations reported in the literature as nutraceuticals do not reach the market due to a lack of more in-depth studies, including clinical trials, or for legal or technical reasons. Lenoir *et al*. (1999) tested the effects of tablets containing three different concentrations of shoot tips from *Hypericum perfuratum* L. on symptoms in patients with mild to moderate depression. The bark of *Phellodendron amurense* Rupr., traditionally used in Chinese medicine, and the peel of *Citrus sinensis* (L.) Osbeck were inserted into pills in order to evaluate their beneficial effects in joint pain; both species contributed to weight loss in tested patients and also an improvement in their health status (Oben *et al*. 2009). The leaves of *Murraya koenigii* (L.) Sprengel and *Tribulus terrestris* L. are traditionally used in India for curry and to treat infertility and impotence, respectively. Sengupta *et al*. (2011) studied the effects of both plant pills in benign prostatic hyperplasia, obtaining satisfactory results.

Type 2 diabetes is increasing worldwide; moreover, the additional health problems related to this disease are also an important concern. Kianbakht and Dabaghian (2013) reported the effects of pills prepared from *Salvia officinalis* L. leaves in patients with type 2 diabetes and hyperlipidemia, describing good results in contrast to the placebo group, and without adverse side-effects. Furthermore, Hosseini *et al*. (2014) proved the effectiveness of pills obtained from *Juglans regia* L. leaves in patients with type 2

diabetes. Genta *et al*. (2009) studied humans given “yacon” syrup obtained from the roots of *Smallanthus sonchifolius* Poepp. & Endl with a high fructooligosaccharide content, demonstrating beneficial health effects in insulin-resistant patients.

Age-related cognitive changes and dementia are also a worldwide concern. *Gingko biloba* L. leaves have been described as being able to affect some neurological properties. Bäurle *et al*. (2009) studied the effects of tablets made from the extract of this plant in mild cognitive impairment; the authors reported the nutraceutical as safe, effective, and acting as an adjuvant to patients who suffer from this illness. There is already on the market a product, Memo®, prepared from *G. biloba* leaves and *Panax ginseng* sp. roots, used against mild cognitive impairment, by slowing the cognitive decline that occurs during the aging process (Yakoot *et al*. 2013). Cognitive wellbeing is also related to stress, depression, and fatigue, and the methods used to treat stress conditions range from a balanced nutritional plan to powerful drugs such as benzodiazepines. Relora® is a pill formulation consisting of a blend of bark extracts of *Magnolia officinalis* Rehder & Wilson and *P. amurense* standardized to honokiol and berberine, respectively, used in the treatment of stress and anxiety; the results achieved in a clinical trial performed by Talbott *et al*. (2013) showed that the combination of these two plants improved a variety of mood state parameters, lowering fatigue and increasing vigor.

Current stress-related diseases are a direct consequence of our modern lifestyle; the human organism produces reactive oxygen species, which are related to higher incidences of cardiovascular, brain, and immune system diseases (Carocho & Ferreira 2013b). Therefore, nutraceutical formulations are also being studied for their antioxidant properties, such as the syrup obtained from the fruits of *Ficus carica* L. and *Prosopis* pods, both widely used in traditional cuisine to prepare desserts and sweets (Puoci *et al*. 2011; Quispe *et al*. 2014). Furthermore, the syrup and pills prepared from *G. biloba* leaves, known for their action against degenerative neurological diseases, as previously mentioned, but also for their action in the cardiovascular system and cerebral vascular activity, were studied for their antioxidant capacity,

showing higher activity than the corresponding infusion and extract; this higher activity was attributed to the highest content in phenolic compounds (Pereira *et al*. 2013a). Pereira *et al*. (2013b, 2014) also tested different nutraceutical formulations (pills and syrup) prepared using *Cynara scolymus* L., *Cochlospermum angolensis* Welw., and *S. marianum*, known for their capacity to prevent oxidative stress and liver disease, in terms of antioxidant and antihepatocellular carcinoma activities; the synergistic effects between these nutraceuticals (mixtures) were also assessed, showing many advantages over individual components.

The bioavailability of nutraceutical formulations is also a hot research topic as metabolic reactions can decrease their bioactive properties. There are already some studies in this direction, such as the one conducted by Matthias *et al*. (2007) on liquid (alcoholic solution) and tablet formulations prepared with *E. purpurea* and *E. angustifolia* roots. Alkylamides, found in both species, were used as target compounds to evaluate the nutraceuticals' bioavailability; these compounds were rapidly and easily absorbed in both formulations. A similar study was performed with tablets prepared with red wine grape extracts made from *Vitis vinifera* L. cv. *Cabernet sauvignon* and *Vitis vinifera* L. cv. *Merlot*, in order to assess the bioavailability of resveratrol; however, in this case the bioavailability was higher in the natural matrix than in the nutraceutical formulation (Ortuño *et al*. 2010).

Nutraceuticals can also combine plant-based principles with other natural matrices such as mushrooms. A good example is ASHMI™, a pill formulation use in asthma treatment, containing the plants *Sophora flavescens* Aiton and *glycyrrhiza uralensis* Fisch. (root aqueous extracts) and the mushroom *Ganoderma lucidum* (Curtis) P. Karst. (fruiting body aqueous extracts) (Kelly-Pieper *et al*. 2009). Wong *et al*. (2004) also studied the effects of *Coriolus versicolor* (L.: Fr.) Quél. and *Salvia miltiorrhiza* Bunge pills (polysaccharides extract) on the improvement of cellular immunity in healthy subjects, which proved to be effective and without adverse effects.

9.4 Wild Plant-Based Drugs

9.4.1 From the Bioactive Phytochemical to the Active Principle

Plants have been used as medicine by humans for thousands of years, since their first use as teas, tinctures, poultices, etc. to the isolation of morphine from opium in the early nineteenth century. Since then, administration methods have changed drastically (Balunas & Kinghorn 2005; Newman *et al*. 2000). Today, there are many sources of new bioactive compounds, including plants, bacteria, fungi, and marine organisms; in fact, from 1981 to 2002, 61% of the 877 new small molecule chemical compounds were derived from natural products, and in specific therapeutic areas (antibacterial, antifungal, antiparasitic, and antiviral treatments), these compounds have provided 70% of total drugs (Cechinel-Filho 2012). There are six classes of compounds that result from botanical sources:

- bioactive compounds that are used directly as drugs, as in the case of digoxin, used for heart conditions
- bioactive compounds with structures that may act as lead compounds to more potent drugs, for instance, paclitaxel, a mitotic inhibitor used in cancer chemotherapy
- chemophores, which are cells that transduce energy, and may be converted into druggable compounds
- pure phytochemicals that can be used as markers to standardize crude plant material
- phytochemicals that can be used as pharmacological tools
- herbal extracts as botanical drugs or green tea extracts (Katiyar *et al*. 2012).

Although there are numerous classes of compounds and methods of obtaining them, the pharmaceutical industry faces unprecedented challenges, with fewer compounds being found, tested, and released to the public. Typically, after *in vitro* assays showing bioactivity of a specific compound, it may start

preclinical studies on animal models followed by a “New Drug Application” addressed to the FDA (USA) and EFSA (EU). If approved, the human studies take place, divided into three phases with escalating numbers of participants to determine the toxicity, side-effects, and other effects not detectable in animal models. The ideal approval process of a new drug is hardly ever linear, and several drawbacks ensue, meaning that several years to some decades may elapse before a compound is marketed as a drug (FDA 2014; Paul *et al.* 2010). Compounds leading to hypothetical drugs must achieve suitable solubility and chemical stability, demonstrate effectiveness in animals (adequate pharmacological profile) and satisfactory bioavailability (with a good half-life), the interactions with cytochrome p450 (CYP450) must be clarified and finally, there must be no obvious toxicity (Cechinel-Filho 2012).

With the reduction of new compounds appearing as potential drugs, humans have once again turned to Nature in order to mitigate the relative void of combinatorial chemistry to find new compounds (Phillipson 2007). The quest for compounds in plants can be carried out in many ways.

- Random selection followed by chemical screening (simple tests that may lead to false positives and false negatives, rendering conclusions difficult to assess and the class of compounds responsible for the activity impossible to specify).
- Random selection followed by one or more biological assays (carried out in animals or *in vitro* assays that screen high volumes of plant species in order to find new drugs).
- Follow-up of biological activity reports (reports of plant extracts with interesting biological activity, which were not studied for their active principles).
- Follow-up of ethnomedical (traditional medicine) uses of plants – plants used in traditional systems like Ayurveda, Unani, Kampo, and traditional Chinese medicine which are not seen as credible by Western scientific methods and are harder to assess, but their undeniable results in many illnesses are impossible to overlook. Herbalism, folklore, and shamanism, which are also viewed with scepticism, are also considered due to their strong reliance on endemic plants.

- Use of databases (large literature sources systematically organized that allow correlation of ethnomedical practices with experimental biochemical and pharmacological activities or to identify plants with multiple effects) (Fabricant & Farnsworth 2001).

To achieve the final compound, a large number of molecules must be extracted from the medicinal plant through various methods.

- Percolation, used for poorly soluble plants or when the price of the plant is relevant. The matrix is placed in a container with solvent flowing through it.
- Countercurrent extraction is obtained by moving solvent through the raw plant in countercurrent.
- Supercritical fluid extraction is carried out by placing the raw plant in a container and filling it with supercritical fluid until the pressure and temperature rise by a considerable amount. These conditions help the fluid to achieve a very high solubility capacity, extracting the compounds of interest.
- Microwave-assisted extraction relies on microwaves that extract compounds more selectively and rapidly while depending less on solvents.
- Maceration is the process of placing the raw plant in a container for different periods of time, while kinetic maceration uses the same process but the mixture is maintained under constant stirring.
- Turbo-extraction uses a cold solvent at high sheer forces, which leads to particle reduction, cell disintegration, and temperature increase.
- Decoctions and infusions rely on hot water as the extractor. Infusions are prepared by adding the plant to boiling water, and maintaining it for 5–10 minutes, while decoctions are prepared by adding the plant to cold water and heating it until it boils, maintaining it for 5–10 minutes.
- Soxhlet extraction relies on cycles of extraction within a glass chamber in which the solvent boils and condenses back into contact with the plant. After filling the chamber it is unloaded into a glass recipient that is heated, evaporating the solvent,

only to condense back into the chamber, in a cyclical way.

- Sublimation extraction sublimes the compounds of interest leaving behind impurities which then condense in another chamber.
- Steam distillation relies on steam to carry the compounds from the boiling mixture containing the plant which then condenses.
- Ultrasonic-assisted extraction is used to increase mass transfer between the plant material and a solution by inducing liquid circulation and turbulence (Cechinel-Filho 2012; Sarker & Nahar 2012; Sticher 2008).

After extraction, the solutions have to be screened to determine their constituents and dereplication (which is the process that recognizes previously studied components that are not important for a screening of new ones) to then prepare for separation and isolation. To separate and isolate the mixtures into their constituents, several methods are used; HPLC is the simplest and can yield results in a short time without needing derivatization steps, although the results can be poor in resolution, and confusing. Ultra high-pressure liquid chromatography (UHPLC) is an improvement on HPLC by enhancing the resolution and throughput for rapid fingerprinting of crude extracts. Liquid chromatography coupled to a photo diode array (LC-PDA) detector is another add-on to a HPLC by allowing a view of the UV spectra, which is useful for detecting compounds with characteristic chromophores. HPLC-MS is HPLC that is coupled to a mass spectrometer, aiding detection, quantification, and identification by providing at the same time a chromatographic (retention times) and a mass spectrometric (m/z) dimension. HPLC-NMR is one of the strongest HPLC methods used to separate compounds. It has the advantage of not relying on commercial databases for spectral comparison, like HPLC-MS. HPLC-NMR provides structural information or even stereochemical information, as well as detection of any hydrogen-containing compounds. LC-SPE-NMR uses a solid-phase extraction coupled to a HPLC and finally a NMR detector, and allows the NMR detection after HPLC separation by either trapping the peaks on SPE or by HPLC microfractionation, drying, and reinjection of the concentrated peak in a microflow capillary

LC-NMR probe. Microflow NMR and cryogenized probes are derivations of this technique (Cechinel-Filho 2012).

It is incontestable that medicinal plants provide unlimited opportunities for new drug discovery because of the unmatched availability of chemical diversity. Nevertheless, since bioactive phytochemicals occurring in plant materials consist of multicomponent mixtures, their extraction, separation, and isolation still create problems. In fact, extraction techniques can negatively affect the integrity of active principles, and practically all of them have to be purified by the combination of several chromatographic techniques or various other purification methods. Thus, it is expected that improvements in these methods will allow us to overcome some of the current limitations, as well as driving the development and introduction of new technologies.

9.4.2 Common Formulations in Drugs from Plant Origin

Drug development has evolved steadily since it first began as part of traditional medicine, and today more and more plant compounds are used as precursors, prototypes, and probes in drug production (Ramawat & Mérillon 2008). Depicted in Table 9.3 are some of the most important drugs either developed using compounds derived from plants, or synthetic ones that were inspired by them, along with the plant from which they were first isolated and the illnesses they are used for. The recent change of attitude from big pharmaceutical companies, which are starting to look for natural compounds, has been a major tonic in the industry, helping to develop new drugs. The applications of natural compounds for human health are endless, and considering that currently only one-quarter of flowering plants is used, there is hope of finding treatments and solutions for many patients around the world (Lange 2004).

Table 9.3 Drugs derived from natural products.

Plant of origin before	Used part	Active principle	Application	Reference

modification				
Artemisia annua L.	Aerial parts	Artemisinin	Antimalarial	Phillipson 2007
Atropa belladonna L.	Aerial parts	Tiotropium	Chronic obstructive pulmonary disease	Balunas & Kinghorn 2005
	Aerial parts	Atropine	Mydriatic agent, antispasmodic	Ramawat & Mérillon 2008
Betula spp. L.	Bark	Betulinic acid	Melanoma, anticancer, antimalarial, anti-HIV, anthelmintic, antiinflammatory, antiretoviral	Balunas & Kinghorn 2005; Ramawat & Mérillon 2008,
Callistemon citrinus Curtis	Aerial parts	Nitisinone	Tyrosinemia	Balunas & Kinghorn 2005
Calophyllum lanigerum W.	Aerial parts	Calanolide	Anti-HIV	–
Camptotheca acuminata Decne	Bark and stem	Camptothecin	Anticancer	Phillipson 2007
	Bark and stem	Topotecan	Metastic ovarian cancer	–
	Bark and stem	Irinotecan	Colorectal cancer	–
	Bark and stem	Exatecan	Anticancer agent	Balunas & Kinghorn 2005
Capsicum spp. L	Fruit	Capsaicin	Osteoarthritis, psoriasis, diabetic,	Ramawat & Mérillon 2008

			neuropathy	
Catharanthus roseus L.	Aerial parts	Vindesine	Leukemia and lung cancer	Phillipson 2007
	Aerial parts	Vinorelbine	Breast cancer	–
	Aerial parts	Vinflunine	Anticancer agent	Balunas & Kinghorn 2005
Chondrodendron tomentosum Ruiz & Pavón	Aerial parts	Tubocurarine	Neuromuscular blocking agent	Phillipson 2007
Combretum caffrum (Eckl. & Zeyh.) Kuntze	Aerial parts	Combretastatin A4 phosphate	Anaplastic thyroid cancer	Ramawat & Mérillon 2008
Dioscorea genus	Tubers	Diosgenin	Contraceptive	–
Erythroxylum pervillei Bail	Stem bark	Pervilleine A	Epidermoid cancer	Balunas & Kinghorn 2005
Euphorbia peplos L.	Sap	Ingenol 3-angelate	Skin conditions	Ramawat & Mérillon 2008
Galanthus woronowii Losinsk.	Bulbs and flowers	Galantamine	Alzheimer's	Balunas & Kinghorn 2005
Galega officinalis L.	Aerial parts	Guanidine derivatives	Type 2 diabetes	Ramawat & Mérillon 2008
Glycine max L. Merril	Aerial parts	Phenoxodiol	Cervical, ovarian, prostate, renal, and vaginal	–

			cancer	
Huperzia serrata Thunb. (Ex Murray) Trevis	Aerial parts	Huperizine	Alzheimer's	–
Illicium verum Hoof f.	Fruit	Oseltamivir phosphate (Tamiflu)	Influenza	–
Panax ginseng L.	Aerial parts	Protopanaxadiol	Apoptotic effect in cancer cells	–
Papaver somniferum L.	Seed pods	M6G	Pain medication	Balunas & Kinghorn 2005
	Seed pods	Apokyn	Parkinson's	Ramawat & Mérillon 2008
Podophyllum peltatum L.	Root	Etoposide	Small cell lung cancer, lymphomas, testicular cancer	Phillipson 2007
	Root	Teniposide	Brain tumors	–
Physostigma venenosum Balf.	Seed	Physostigmine	Parkinson's	–
Plectranthus barbatus Andrews	Aerial parts	Colforsin daropate	Anticancer	Butler 2005
Taxus brevifolia Nutt.	Bark	Taxol	Anticancer chemotherapy	Phillipson 2007
	Bark	Taxotere	Breast cancer and nonsmall	–

cell lung cancer (adjuvant)

The WHO reports that over 21 000 plant taxa are used for medicinal purposes, although this number does not include cosmetics, spirits, and aromas (FAO 2002; Lange 2004). Roughly 80% of developing countries depend on plant-based drugs, although the WHO suggests that in the near future a similar percentage of the entire world population will depend on them. Furthermore, 30% of the drugs sold worldwide contain products derived from plants (FAO 2005).

Of the global trade in medicinal plants, it is hard to know how much is represented by wild or cultivated ones. Although the pharmaceutical industry has isolated a large number of bioactive compounds from wild plants (edible and medicinal), there are considerable disadvantages in harvesting wild medicinal plants rather than cultivating them for industrial drug development. The pharmaceutical industry mainly uses cultivated plants as primary material, despite the expensive domestication and cultivation process, in order to obtain a standard and well-known source of the active principle, in the necessary amounts for industrial-level processing. Moreover, there are some disadvantages related to wild plant gathering, including uncontrolled harvest that leads to extinction of the plant and erosion of the ecosystem. Other problems include poor knowledge about the biology of the plants, little or no inventory, ownership conflicts of the harvest zones, and scarce income due to overharvesting. Cultivation in small farms and households or in large and extensive production facilities could be an alternative, although the disadvantages are still great, due to the large investments needed, the reduction of incentive to conserve native ecosystems, devaluation of wild plants, reduction of genetic diversity and the risk of the introduced plant becoming an invasive species (FAO 2002).

9.4.3 Wild Plant-Based Drugs for Different Therapeutic Targets

Wild plant-based drugs are everywhere; the definition of a drug is

quite vague, encompassing all “chemical substances used in the treatment, cure, prevention, or diagnosis of disease or used to otherwise enhance physical or mental well-being.” In this way, all molecules used by any type of medicine, modern or traditional, could be classed as drugs. To narrow down the results, only drugs used and approved in Western modern medicine are considered here, otherwise the list would be endless, although alternative medicines are quite well documented (Ahmad *et al*. 2006; Hawkins 2008; Osbourn & Lanzotti 2009; Trivedi 2009).

Medicinal plants represent 25% of prescription drugs in modern medicine. Of the 3000 plants traded for medicinal purposes, only 900 are cultivated, which means that 70–80% of the whole market depends on wild collection (Hawkins 2008). The conservation of habitats of these plants is the responsibility of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which is the most important source of information on wild medicinal plants in use. *Galanthus* spp. L., an herbaceous plant endemic to the northern hemisphere, is the source of galanthamine, an approved drug used against Alzheimer’s disease (Heinrich & Teoh 2004). *Taxus brevifolia* Nutt., the conifer that is the source of the anticancer agent Taxol®, also known as paclitaxel, is another success story of the medicinal power of plants, although it has endangered some cultivars of the tree. The alkaloid colchicine, derived from *Colchicum autumnale* L. corms, is used for the treatment of gout, under the name Colcrys (Romano 2013). The treatment of cardiac diseases also depends on compounds derived from wild medicinal plants, including digoxin, a cardiac glycoside extracted from the herb *Digitalis lanata* Ehrh. It is also marketed under the names Lanoxin®, Lanoxicaps®, Cardoxin® and Digitek®, among others (Hawkins 2008). The cinchona tree, *Cinchona officinalis* L., endemic to South America, is a natural source of quinine, a known antimalarial alkaloid that is used against this disease in modern medicine. There are reports of other uses of this molecule, which have recently been investigated (Christoforidis 2014). *Camptotheca acuminata* Decne is a tree native to China and Tibet which is rich in an alkaloid called camptothecin, used as an anticancer agent (Gaur *et al*. 2014). These examples illustrate some of the illnesses that can be cured or attenuated with wild plant compounds.

The endless combination of compounds found in nature that may have application in medicine provides hope for treatments of illnesses that have not yet been controlled or cured. The search to find new compounds continues at a steady pace and technology keeps lending precious help to this quest. Wild medicinal plants are today still as valuable as they were in the pre-modern medicine era. However, the pursuit of bioactive compounds should never overlook the habitats and wellbeing of the species. Research should continue to try and cultivate the plants that are not yet fit to be intensively grown, therefore reducing dependency on wild plants. But while there is no alternative, mankind should harvest them from nature, but always ensuring their continuity for generations to come.

9.5 Conclusion

Functional foods and nutraceuticals have been reported as one of the top trends of today’s food industry. Apart from the naturally occurring functional foods, the development of new functional foods, nutraceuticals, and drugs based on plants is an active and very promising area of research, indispensable for the substantiation of health claims and benefits. The characterization of plant ingredients by advanced technologies, standardization of human clinical trials, and the use of emerging methodologies are crucial strategies for the development of new functional products and drugs. Additionally, the degree of acceptance and awareness of functional foods and nutraceuticals by consumers, the association between manufacturers and academic researchers, and the effects of new regulations for nutrition and health claims are crucial factors for future market evolution. Despite all the potential of these products to prevent diseases and promote human health, health professionals, nutritionists, and regulatory toxicologists should work together to plan appropriate regulation to provide the ultimate health and therapeutic benefit to humans.

However, due to the rising demand for plant-based functional foods, nutraceuticals, and drugs in higher quantities to promote health, longevity, and quality of life, wild harvested medicinal plants are taking on an increasing role and many of them have

become endangered due to irresponsible collection, associated with economic interests. Therefore, the cultivation of these species is an alternative that needs to be taken into account. Furthermore, the next phase of market growth depends on valid scientific research for new product technologies, patents, more effective branding, and trademark strategies in product manufacture and international regulatory compliance.

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10

Nuts: Agricultural and Economic Importance Worldwide

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10.1 Introduction

Several tree nuts have agronomic and economic importance: almonds, Amazonia nuts (Brazil nuts), cashews, chestnuts, hazelnuts, macadamias, peanuts, pecans, pine nuts, pistachios, and walnuts are just some examples (Figure 10.1). While some of these nuts have local or regional importance, others have worldwide significance, which is the case for almonds, chestnuts, hazelnuts, and walnuts.

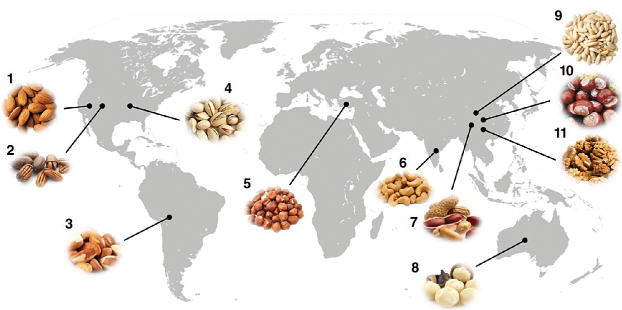

Figure 10.1 Main nuts produced worldwide and main producers in 2014–15 season (1, almonds; 2, pecans; 3, Brazil nuts; 4, pistachios; 5, hazelnuts; 6, cashews; 7, peanuts; 8, macadamias; 9, pine nuts; 10, chestnuts; 11, walnuts) (ICN 2015).

The production of nuts worldwide is increasing. In the season 2014–15, according to the International Nut and Dried Fruit Council (INC), more than 3.6 million tons of nuts (shelled) were produced (INC 2015). This is 8.5% higher than production from

the 2013–14 season and 56% higher compared to 2004–05 (INC 2015). Moreover, production is expected to grow in the coming years. The USA, the main producer of nuts worldwide, estimates a continuous increase in production until 2024, with an expectation of export values of nuts in 2024 of over US$13 745 million (USDA 2014).

Regarding consumption, high- and middle-income economies are increasing their intake while in low-income economies consumption is more unstable.

This chapter presents a detailed agronomic and economic perspective based on almonds, chestnuts, hazelnuts, and walnuts. The research is based on international sources of statistics, from the Food and Agriculture Organization (FAO) and INC, mainly from the 2000–13 period. We discuss the current situation and recent evolution in world production, trade and consumption of almonds, chestnuts, hazelnuts, and walnuts, including top producers, importers, exporters, and main consumer countries.

10.2 Almond

Almond (*Prunus dulcis* Mill. D.A. Webb) is mainly cultivated in regions with temperate and subtropical climate conditions. Almonds are classified into two categories: sweet and bitter. The sweet almond is *Amygdalus communis* L. var. *dulcis* while the bitter almond is *A. communis* L. var. *amara*.

Worldwide, for domestic consumption and for trade purposes, the sweet almond is more popular, while the bitter almond is commonly used for industrial purposes, as a flavor included in several food products, among them alcoholic beverages. This nut is used in several forms: whole (blanched or natural), sliced (blanched or natural), slivered, diced, as flour, as a paste, and as a vegetable oil. The diverse food products and healthy properties inherent in almonds' chemical composition (Chen *et al.* 2006) attract consumers' attention. Almonds are a good source of lipids, proteins, carbohydrates, minerals, and vitamins (Yada *et al.* 2011),

as well as a source of minor compounds with bioactive characteristics (Barreira *et al*. 2008a; Monagas *et al*. 2007). This has boosted the world production of almonds (Figure 10.2). Figure 10.2 shows that between 2000 and 2013, the harvested area was reduced around 2.3% but production (shown in detail in Table 10.1), and consequently the yield, has increased tremendously since 2000.

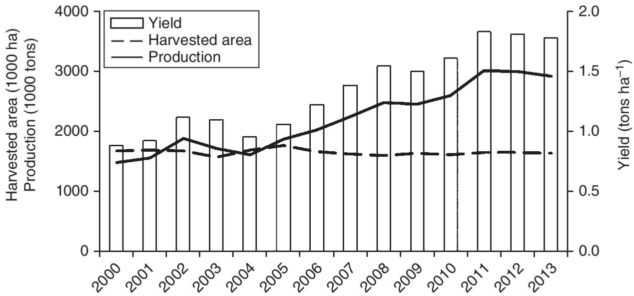

Figure 10.2 Evolution of almond (with shell) production, harvested area, and yields from 2000 to 2013 (FAOSTAT 2015).

Table 10.1 World production and trade of almonds (2000–12 period) (elaboration based on FAOSTAT data; FAOSTAT 2015).

	Production					World trade[d,e]		
Year	**Volume[a] (tons)**	**Gross value[b] (US $1000)**	**Unit price[c] (US$)**	**Implicit price index (2000 = 100)**	**Volume index (2000 = 100)**	**Volume (tons)**	**Value (US $1000)**	**Unit price (US$)**
2000	1 479 636	1 967 780	1.33	100.00	100.00	268 963	777 347	2.89
2001	1 560 612	1 988 350	1.27	92.21	105.47	299 614	796 499	2.66
2002	1 877 574	2 585 820	1.38	97.82	126.89	346 694	993 352	2.87
2003	1 717 743	3 481 850	2.03	137.92	116.09	361 139	1 247 275	3.45
2004	1 617 261	4 687 830	2.90	189.75	109.30	364 774	1 652 910	4.53

2005	1 864 411	5 741 610	3.08	236.66	126.00	344 764	2 304 176	6.68
2006	2 024 753	5 281 290	2.61	182.78	136.84	399 800	2 369 507	5.93
2007	2 253 125	6 221 740	2.76	164.12	152.28	436 905	2 264 590	5.18
2008	2 479 892	6 021 300	2.43	140.34	167.60	463 137	2 194 737	4.74
2009	2 456 874	5 865 640	2.39	152.76	166.05	533 660	2 118 056	3.97
2010	2 597 441	7 372 880	2.84	167.05	175.55	541 919	2 548 472	4.70
2011	3 013 215	10 153 890	3.37	185.50	203.65	601 779	2 960 270	4.92
2012	3 004 847	10 879 650	3.62	198.11	203.08	639 885	3 453 685	5.40

a Production relates to almonds in the shell or in the husk.

b Current prices, calculated without any deductions for seed.

c Price received by farmers for 1 kg of product.

d Shelled almonds, amount related to the average of exports and imports.

e Export values are mostly reported as free-on-board (FOB) (i.e. insurance/transport costs are not included) and import values mostly as cost-insurance-freight (CIF) (i.e. insurance/transport costs are included).

Improvements in efficiency and technology are the main factors for the tremendous increase of almond yields over the years. Other factors, such as advances in tree varieties, planting patterns, improvements in mechanization and orchard agronomy, together with irrigation, have also encouraged the increase in almond production and yield.

According to the most recent statistics from the FAO (FAOSTAT 2015), worldwide almond production reached about 2 917 894 tons in 2013, being cultivated and spread all over the world (Figure 10.3). The top 10 producers accounted for more than 90.5% of almond production in 2013. Nevertheless, almond

production is mainly concentrated in the United States of America (USA, California) with more than 62% of world production, followed by Australia (5.5%) and Spain (5.1%) (see Figure 10.3).

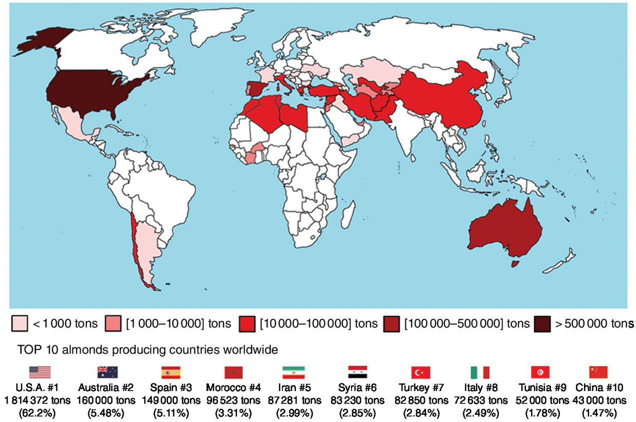

Figure 10.3 Worldwide almond with shell production (tons) and top 10 producers for 2013 (FAOSTAT 2015).

In the USA, mainly in California, about 10 almond varieties count for about 70% of the production: Nonpareil, Carmel, Butte, Padre, Mission, Monterey, Sonora, Fritz, Price, and Peerless (Almond Board of California 2015). Half of USA almond production is usually intended for domestic consumption and the remainder for foreign markets, thus making the USA the world's main exporter and consumer of this nut. In Australia, the most popular varieties are Nonpareil, Carmel, Price, and Peerless. Australian almonds are mainly consumed domestically, with only 25% of the country's annual production being exported. In Spain, the varieties Marcona, Largueta, Planet, Communes or Valencianas, and Mallorca are mostly grown. Spain is an important producer and also a major consumer of almonds, since almond is an important element in the traditional Mediterranean diet, either as an appetizer or an ingredient in the confection industry, such as traditional nougat, marzipan, and pastries. Additionally, Spain is a major processor of almonds; accordingly, Spanish international trade in almonds is very intense (Spain is the second largest importer and exporter of almonds). As a result, Spanish national production is insufficient to satisfy its domestic and foreign demand, and this country presents a deficit trade balance. This deficit, however, is not verified in

terms of value, since being an almond-processing country, Spain adds value to its almond exports.

10.2.1 Evolution of Almond Production and Trade Facts

In 2012, world production of almonds with shell reached 3 million tons, corresponding to US$10 880 million at current prices (see Table 10.1). This is 103% higher than in 2000, an annual average growth rate of 6.4%. This evolution was associated with a price increase of around 98% (7.5% a year, on average). Hence, overall production value increased 453% at current prices, in the 2000–12 period. These findings indicate the increasing market valorization of almond production, despite some loss of momentum during 2006–09, with an overall increase of more than 172.5% of unit price per kg over the decade. These results are also confirmed by the evolution of consumption with an average yearly growth rate of over 7% in the 2004–12 period.

World trade in shelled almonds in 2012 was almost 640 000 tons, worth US$3.45 million. Compared to 2000, this represents an increase of 138% in volume, and 344% in value. Table 10.2 presents the world top exporters and importers of shelled almonds.

Table 10.2 Top exporters and importers of shelled almonds (three year average) (elaboration based on FAOSTAT data; FAOSTAT 2015).

Exports[a]				**Imports**[b]			
Volume (tons)		**Value (US $1000)**		**Volume (tons)**		**Value (US $1000)**	
USA	440 234	USA	2 137 431	Germany	79 978	Germany	379 834
Spain	61 206	Spain	341 997	Spain*	63 244	China	268 032
China	24 458	China	100 643	China	56 181	Spain	267 101
Australia	17 710	Australia	86 424	UAE**	36 671	UAE	215

							973
Netherlands	11 449	Germany	58 126	Italy	31 997	Italy	161 932
Others	59 795	Others	361 891	Others	306 132	Others	1 545 491

a Export values are mostly FOB.

b Import values are mostly reported as CIF.

* Processing country.

** United Arab Emirates (UAE), Transit country.

The USA is the main exporter of shelled almonds, with over 440 000 tons per year (see Table 10.2), approximately 70% of the world export total in volume and value. The main export destinations of USA almonds are Spain, Germany, and China, and together these countries represent almost 40% of USA exports. Spain is the second biggest exporter with about 61 000 tons per year, approximately 10% of world trade; 60% of Spanish exports are destined for Germany, Italy, and France.

Imports are less concentrated, Germany, Spain, and China being the main importers with 80 000, 63 000, and 56 000 tons per year, respectively. This represents around 14%, 11%, and 10% of the volume and 13%, 9%, and 9%, of the value of world imports, respectively.

10.2.2 Consumption of Almonds Worldwide

Table 10.3 reports the evolution of almond consumption worldwide from 2004 to 2012. World almond consumption is increasing (71.1% from 2004 to 2012); in 2012 the consumption of shelled almonds per capita was 135 g (INC 2013), the highest value ever recorded.

Table 10.3 Shelled almond consumption (tons) in the period 2004–12 (elaboration based on INC) (INC 2009, 2013).

Country	2004	2005	2006	2007	2008	2009	2010	2011	2012	Variation Overall	Mean
USA	164	152	183	193	207	211	234	253	269	63.7%	6.6%

	382	407	840	412	701	752	781	294	061		
Germany	56 917	56 828	61 169	62 415	63 136	60 972	70 282	71 729	71 516	25.6%	3.0%
Spain*	29 801	65 517	59 734	44 594	61 760	42 815	43 305	61 649	57 664	93.5%	6.3%
Italy	27 470	36 684	34 958	40 028	29 252	28 583	29 333	39 915	37 130	35.2%	5.7%
China	7073	7 679	12 654	7805	16 305	24 476	28 070	36 103	34 810	392.2%	9.2%
Australia	5054	11 688	11 952	21 282	29 423	33 213	25 307	34 369	30 606	505.6%	3.0%
France	23 800	27 957	23 207	27 616	25 415	29 337	29 712	28 956	30 309	27.3%	3.8%
Japan	24 152	20 233	22 171	24 149	19 240	23 390	21 023	21 777	25 932	7.4%	2.0%
Canada	13 538	12 419	14 768	18 560	18 911	20 146	20 734	24 817	23 800	75.8%	7.9%
Turkey	13 664	15 458	15 476	10 226	12 570	16 114	21 524	22 775	23 172	69.6%	8.9%
WORLD	543 146	588 955	641 763	660 188	685 433	735 699	800 962	908 026	929 472	71.1%	7.0%

* Processing country.

The USA is responsible for almost 30% of world consumption of shelled almonds. The rise in world consumption was mainly driven by domestic demand from the USA, Spain, China, and Australia. These four countries jointly are responsible for half of the increase in world consumption of shelled almonds. From 2004 to 2012, the consumption of shelled almonds in China rose 392.2%, while in Australia the increase was even higher, at 505.6% (see Table 10.3). When consumption is reported by per capita/year, in 2012 higher consumption was verified in Australia, Tunisia, and Greece, with 1.498, 1.227, and 1.100 kg/capita/year respectively (INC 2013).

10.3 Chestnut

Chestnuts worldwide include three species: Chinese chestnut (*Castanea mollissima* Blume), European chestnut (*C. sativa* Mill.), and Japanese chestnut (*C. crenata* Siebold & Zucc.). Chestnuts are normally sold to the consumer with shell, in order to increase their shelf-life. However, they can be sold without shell, mainly frozen, throughout the year. Chestnuts are normally used as a whole or as an ingredient (as a paste) to be included in several dishes or for sweets, cakes, and dessert preparations. A very popular preparation is the "marron glacé," eaten as a whole or as an ingredient in desserts. The nutmeat can be consumed raw, boiled, cured or roasted (Nazzaro *et al*. 2011).

Chestnuts are an excellent source of carbohydrates, with a caloric intake around 400 kcal/100 g (Vasconcelos *et al*. 2010). Fat composition of chestnuts is mainly polyunsaturated fatty acids, due to the high content of linoleic acid; there are also appreciable amounts of oleic acid and low levels of saturated fatty acids (Vasconcelos *et al*. 2010). Even after culinary processing (boiling and roasting), chestnuts retain appreciable amounts of minor components, such as phenolic compounds (Gonçalves *et al*. 2010) and vitamins A, B_1, B_2, B_3, B_5, B6, C, and E (Vasconcelos *et al*. 2010). These minor components are responsible for the antioxidant potential verified in the nutmeat (Barreira *et al*. 2008b). A more detailed description on these aspects is given in Chapter 13.

Chestnut production is beating records each year. For the first time in history, chestnut production passed the 2 million ton mark in 2012, and in 2013 another production record of 2 009 000 tons was reported (FAOSTAT 2015), the highest production ever recorded (Figure 10.4).

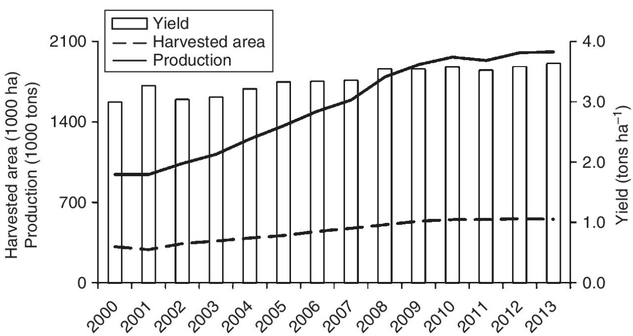

Figure 10.4 Evolution of chestnut production, harvested area, and yields from 2000 to 2013 (FAOSTAT 2015).

Production was also boosted by the increase in harvested area, which has steadily risen in recent years, reaching 552 478 ha worldwide in 2013 (FAOSTAT 2015). Yields are also increasing continuously, to 3.63 tons per ha in 2013 (see Figure 10.4). Distribution of world chestnut production in 2013 is represented in Figure 10.5.

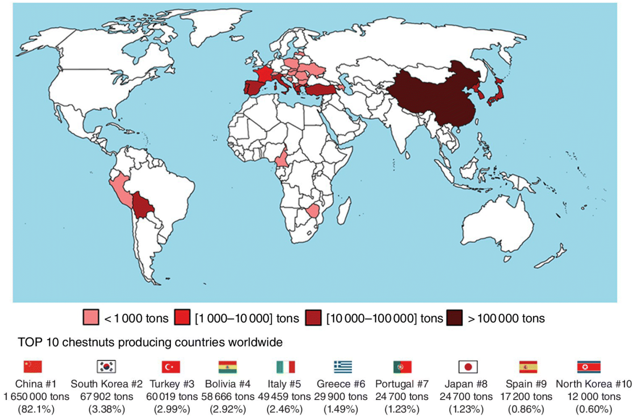

Figure 10.5 Worldwide chestnut production (tons) and top 10 producers for 2013 (FAOSTAT 2015).

China is the main producing country with more than 82% of world chestnut production. South Korea and Turkey are the second and third producers with around 3.4% and 3.0% respectively (see Figure 10.5). In China and South Korea, the main cultivated chestnut variety is *C. mollissima*, while in Turkey the main variety is *C. sativa*. The top 10 chestnut-producing countries account for about 99.3% of worldwide production (FAOSTAT 2015). Despite being the world's main producer of chestnuts, China only exports approximately 2% of its annual production. South Korea's and Turkey's harvests are also primarily destined for domestic consumption, with only 15% being sold in foreign markets. In contrast, Portugal and Spain export almost half of their annual chestnut production, mainly to supply French and Italian chestnut-processing factories and Brazilian markets. Italy exports about 40% of its production, processed and fresh, mainly to France, Switzerland, Germany, and Austria.

10.3.1 Evolution of Chestnut Production and Trade Facts

According to FAO statistics (FAOSTAT 2015), in 2012 world production of chestnuts was around 2 million tons, corresponding to US$4 751 000 at current prices (Table 10.4). This represents a growth of 112% from 2000 (an average annual rate of more than 6.5%). This remarkable increase was associated with an implicit prices index of 260.45, and consequently a growth rate of chestnut production value over 400%. Moreover, Table 10.4 shows that although chestnut production experienced a decrease in market value in the early years, there has been a significant recovery in recent years, in terms of both unit price production and world trade unit prices.

Table 10.4 World production and trade of chestnuts (2000–12 period) (elaboration based on FAOSTAT data; FAOSTAT 2015).

	Production					World trade[d,e]		
Year	**Volume[a] (tons)**	**Gross value[b] (US $1000)**	**Unit price[c] (US$)**	**Implicit price index (2000 = 100)**	**Volume index (2000 = 100)**	**Volume (tons)**	**Value (US $1000)**	**Unit price (US$)**
2000	943 234	947 510	1.00	100.00	100.00	99 076	222 952	2.25
2001	943 212	839 530	0.89	88.56	100.00	97 630	199 462	2.04
2002	1 037 692	907 020	0.87	88.43	110.01	103 010	199 803	1.94
2003	1 119 142	1 017 510	0.91	94.62	118.65	105 823	239 856	2.27
2004	1 250 818	1 261 000	1.01	107.20	132.61	110 448	216 526	1.96
2005	1 367	1 449	1.06	113.4	144.95	100	195	1.95

	236	560				241	611	
2006	1 493 156	1 710 340	1.15	122.57	158.30	112 169	206 427	1.84
2007	1 591 247	2 170 090	1.36	146.97	168.70	102 248	226 357	2.21
2008	1 791 430	3 071 300	1.71	187.23	189.92	103 292	243 818	2.36
2009	1 899 255	2 275 930	1.20	131.40	201.36	104 294	228 053	2.19
2010	1 964 598	2 960 790	1.51	164.89	208.28	100 739	246 779	2.45
2011	1 935 232	3 894 360	2.01	221.49	205.17	96 890	263 672	2.72
2012	2 002 810	4 751 680	2.37	260.45	212.33	104 517	325 025	3.11

a Production relates to nuts in the shell or in the husk.

b Current prices, calculated without any deductions for seed.

c Price received by farmers for 1 kg of product.

d Amount related to the average of exports and imports.

e Export values are mostly reported as FOB and import values mostly as CIF.

At the beginning of this century, 10% of world chestnut production was traded internationally. These figures have decreased over time as world trade volume remained relatively stable and, at present, world trade flows involve a little more than 5% of the overall supply (around 100 000 tons per year), representing about US$325 000. Table 10.5 presents the world's top exporters and importers of chestnuts.

Table 10.5 Top world exporters and importers of chestnuts (three year average) (elaboration based on FAOSTAT data; FAOSTAT 2015).

Exports[a]		**Imports**[b]	
Volume (tons)	**Value (US $1000)**	**Volume (tons)**	**Value (US $1000)**

China	36 707	China	79 049	China	15 534	Japan	57 267
Italy	16 800	Italy	76 145	Japan	11 861	Italy	29 058
South Korea	11 189	Portugal	29 508	Italy*	11 381	China	27 694
Portugal	9340	South Korea	28 473	France*	6877	Switzerland	16 340
Spain	7723	Spain	19 426	Thailand	4947	Germany	15 745
Others	19 677	Others	53 312	Others	49 393	Others	124 967

a Export values are mostly FOB.

b Import values are mostly CIF.

* Processing country.

Chestnut world trade flows are concentrated in China, simultaneously the world's main exporter and importer, with over 36 000 tons of outflow and 15 000 tons of inflow per year. China is followed by Italy, with about 17 000 tons of exports and 11 000 tons of imports per year. Together, they are responsible for half the world's exports and 25% of imports.

The leaders of international trade also include South Korea, Portugal, and Spain, which are accountable for around 30% of exports, and Japan, France, and Thailand, responsible for 25% of imports. China exports chestnuts mainly to Japan and Thailand, and imports chestnuts mainly from South Korea. Italy exports mainly to France and Switzerland and imports come from Portugal and Spain.

10.3.2 Consumption of Chestnuts Worldwide

Table 10.6 provides an estimation of chestnut annual consumption based on production, import, and export values (FAOSTAT 2015). Chestnut consumption worldwide increased from 1256 tons in 2004 to 2000 tons in 2012, an increase of 59%, and with an annual growth of 6.1% (see Table 10.6). In regard to consumption per capita, consumption increased from 195 g in 2004 to 282 g per capita in 2012.

Table 10.6 Chestnut consumption (tons) in the period 2004–12

(elaboration based of FAOSTAT 2015).

										Variation	
Country	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**Overall**	**Mean**
China	904 962	1 016 247	1 115 171	1 238 111	1 427 085	1 520 294	1 600 339	1 576 353	1 629 788	80.1%	7.8%
South Korea	57 251	62 087	70 795	67 694	64 950	64 762	57 383	56 450	61 317	7.1%	1.2%
Bolivia	52 758	57 055	55 000	42 801	58 443	55 001	52 200	55 984	57 000	8.0%	2.1%
Turkey	41 798	45 341	50 178	54 949	52 997	59 138	56 345	56 312	52 603	25.9%	3.2%
Italy	24 071	35 690	36 797	38 484	42 780	38 764	36 644	42 048	55 995	132.6%	12.5%
Japan	48 625	42 986	44 559	38 698	37 994	33 939	34 378	30 276	31 159	–35.9%	–5.2%
Greece	19 367	19 417	17 726	15 590	10 140	14 316	20 794	21 257	27 737	43.2%	8.0%
Portugal	26 082	18 734	23 070	17 926	19 376	18 255	16 981	11 584	8 408	–67.8%	–11.4%
France	24 763	13 533	15 612	11 042	17 026	13 490	14 375	12 809	9 300	–62.4%	–7.2%
Spain	3282	[illegible]	[illegible]	[illegible]	[illegible]	11 498	13 175	11 829	8 436	157.0%	21.6%
WORLD	1 256 762	1 376 486	1 497 526	1 589 166	1 795 542	1 894 887	1 962 716	1 935 536	2 000 061	59.1%	6.1%

China is the main chestnut consumer, being responsible for more than 80% of chestnut world demand. The other leading chestnut consumers are South Korea, Bolivia, Turkey, and Italy, with around 10% of world demand, jointly. Chinese per capita chestnut consumption has consistently increased over the time period of 2004–12, from 0.675 kg per capita to 1.157 kg per capita. Italian demand for chestnuts has also grown and its share increased in the same period. Other traditional chestnut-consuming countries such as Japan, Portugal, and France have decreased their demand. Bolivia is the main per capita consumer of chestnuts with 5.431 kg

(2012 estimation), followed by Greece (2.493 kg) and South Korea (1.251 kg).

10.4 Hazelnut

Hazelnut (*Corylus avellana* L.) varieties are classified in three main groups according to their fruit shape. Hazelnuts can be round, spindle shaped or almond shaped. Round hazelnut varieties are preferred for cultivation as they have better characteristics for food industry processing (Ozdemir & Akinci 2004).

Hazelnuts can be consumed raw or dried (blanched or natural). They are used as a paste for cakes and in a diversity of desserts, diced for cake and dessert decoration, and also as a vegetable oil. In addition, hazelnut is a very important ingredient for the chocolate sector in Italy and several other Central European countries, like Austria, Belgium, Germany, Luxembourg, and Switzerland. For instance, in Italy, the Ferrero® Group, responsible for the production of Nutella® and Ferrero Rocher®, is responsible for about 25% of global hazelnut demand.

Hazelnuts are a good source of protein (around 20%) and possess high fat content (from 57% to 63% depending on variety) (Ozdemir & Akinci 2004). One hundred grams of shelled hazelnuts have a caloric value between 649 and 680 kcal, depending on variety (Ozdemir & Akinci 2004). Hazelnuts are also a good source of essential amino acids and minerals (Köksal *et al*. 2006). They also have antioxidant and antimicrobial properties (Oliveira *et al*. 2008) and comparatively to other nuts, they have higher antioxidant properties that have been linked to their phenolic compound content (Delgado *et al*. 2010).

Figure 10.6 reports the evolution of harvested area, production, and hazelnut yield from 2000 to 2013. Harvested area increased about 25% from 2000 to 2013. However, production is relatively unstable which also affects yields. The yield for 2013 was 1.38 tons per ha (see Figure 10.6).

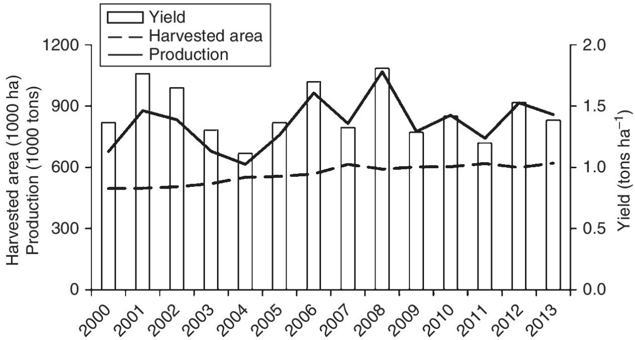

Figure 10.6 Evolution of hazelnut production, harvested area, and yields from 2000 to 2013 (FAOSTAT 2015).

Figure 10.7 represents the worldwide dispersion of hazelnut production and the main producers in 2013. The top 10 producers worldwide represent about 99.4% of production. Around 859 000 tons of hazelnuts were produced in 2013, mainly concentrated in Turkey (63.9%). According to Ozdemir and Akinci (2004), the following varieties of hazelnuts are cultivated in Turkey: Aci, Cavcava, Fosa, Kan, Kargalak, Kus, Mincane, Sivri, Uzunmusa, Yassi Badem, Yuvarlak Badem, Cakildak, Kara, Palaz, and Tombul, but the last four are the major commercial Turkish varieties. Half of Turkish hazelnut production is usually sold abroad, making this country the world's main exporter and the second consumer of hazelnuts, right behind Italy.

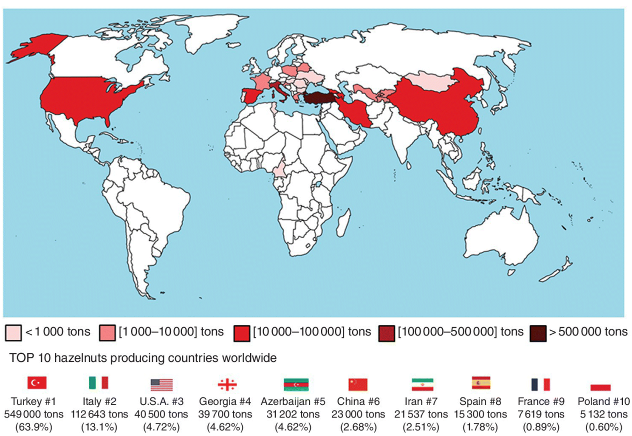

Figure 10.7 Worldwide hazelnut production (tons) and top 10 producers for 2013 (FAOSTAT 2015).

Italy is the second biggest producer with 13.1% of world production. In Italy, the main hazelnut varieties are native: Tonda di Giffoni, Mortarella, San Giovanni, Camponica, Riccia di Talanico, Tonda Bianca, Tonda Rossa, Tonda Gentile delle Langhe, and Santa Maria de Jesus.

Despite being the second largest world producer, Italian hazelnut production is insufficient to satisfy its domestic demand and, in terms of volume, the country presents a trade deficit. This deficit is not verified in terms of value since, as mentioned, Italy is an important processor hazelnuts, especially as input for the chocolate industry, thus adding value to its hazelnut exports.

10.4.1 Evolution of Hazelnut Production and Trade Facts

The worldwide production of hazelnuts is quite unstable, with maximum production values in 2008. Hazelnut production is cyclical, bearing heavily in alternate years, which helps to explain the unstable worldwide production. Table 10.7 details the evolution of production from 2000 to 2012 and the world trade. In 2012, world production of hazelnuts with shell was around 916 000 tons, corresponding to over US$2 600 000 at current prices (see Table 10.7). This production value presents a 150% increase from 2000, essentially because of the prices rising around 89% (implicit price index = 189.08), while volume only increased 35% since 2000, indicating an annual average increase of 4.7%.

Table 10.7 World production and trade of hazelnuts (2000–12 period) (elaboration based on FAOSTAT data; FAOSTAT 2015).

	Production					**World trade**[d,e]		
Year	**Volume**[a] **(tons)**	**Gross value**[b] **(US $1000)**	**Unit price**[c] **(US$)**	**Implicit price index (2000 = 100)**	**Volume index (2000 = 100)**	**Volume**[a] **(tons)**	**Gross value (US $1000)**	**Unit price (US$)**
2000	676 847	1 042 490	1.54	100.00	100.00	175 552	571 157	3.25

2001	878 055	979 590	1.12	72.26	129.73	216 255	624 363	2.89
2002	832 551	904 820	1.09	70.11	123.00	217 476	524 998	2.41
2003	679 466	835 800	1.23	81.06	100.39	202 812	597 791	2.95
2004	615 024	1 046 600	1.70	112.85	90.87	174 513	902 151	5.17
2005	758 629	2 036 320	2.68	176.08	112.08	178 011	1 481 886	8.33
2006	964 015	2 391 920	2.48	162.29	142.43	190 042	1 076 967	5.67
2007	814 500	2 243 850	2.76	182.55	120.34	198 391	1 151 175	5.80
2008	1 069 175	2 937 250	2.75	178.35	157.96	195 618	1 244 145	6.36
2009	775 146	1 860 720	2.40	160.84	114.52	196 138	1 095 580	5.59
2010	854 918	2 229 080	2.61	173.33	126.31	206 885	1 258 987	6.09
2011	742 146	2 147 950	2.89	197.22	109.65	211 401	1 456 621	6.89
2012	915 846	2 603 840	2.84	189.08	135.31	210 784	1 416 406	6.72

a Production relates to nuts in the shell or in the husk.

b Current prices, calculated without any deductions for seed.

c Price received by farmers for 1 kg of product.

d Shelled hazelnuts, amount related to the average of exports and imports.

e Export values are mostly reported as FOB and import values mostly as CIF.

Additionally, despite the relatively modest but steady increase in hazelnut cultivated area (see Figure 10.6) during the period of analysis (an average of 1.9% per year), hazelnut volume and producer's price show substantial annual oscillations. These findings indicate that hazelnut production has experienced somewhat erratic returns, its growth is stuck and its market value,

although significantly increasing overall, is relatively unstable. On the other hand, the world trade price trend of shelled hazelnuts may suggest a recovery of the economic attractiveness of hazelnuts in international markets. Indeed, after the decline in the early years and the abnormal 2004–05 upper peaks, the price of shelled hazelnuts in world trade has shown a steady recovery in recent years, and in 2012 reached US$6.72 per kg, a 19% increase from 2006.

According to the last five years of statistics from the INC, approximately half of shelled hazelnut world production is traded in foreign markets. In 2012, it accounted for approximately 210 000 tons, and over US$1 400 000 (see Table 10.7). The volume of shelled hazelnuts increased over 20% in the 2010–12 period, while value improved more than 148% overall, suggesting a recovering of hazelnut pricing in international markets. Table 10.8 reports the world top exporters and importers of shelled hazelnuts.

Table 10.8 Top world exporters and importers of hazelnuts (three year average) (elaboration based on FAOSTAT data; FAOSTAT 2015).

Exports[a]				**Imports[b]**			
Volume (tons)		**Value (US $1000)**		**Volume (tons)**		**Value (US $1000)**	
Turkey	152 463	Turkey	995 992	Germany*	60 068	Germany	417 490
Georgia	14 104	Italy	98 995	Italy*	29 942	Italy	209 311
Italy	13 877	Georgia	84 218	France	20 284	France	139 380
Azerbaijan	10 533	Azerbaijan	46 044	Russia	13 044	Canada	78 080
Germany	4490	Germany	34 691	Belgium*	10 853	Belgium	75 887
Others	12 624	Others	82 465	Others	77 096	Others	492 124

a Export values are mostly FOB.

b Import values are mostly reported as CIF.

* Processing country.

Turkey is the main exporter of shelled hazelnuts worldwide, with over 152 000 tons per year, approximately 73% of the world export in volume and value, half of which goes to Italy, France, and Germany. Georgia comes in second place with about 14 000 tons per year, corresponding to approximately 6% of exports, whose main destination is Germany. Italy is close, with almost the same volume of exports but with higher export income. Imports are concentrated in Germany, Italy, and France and altogether, they represent more than half of world imports in volume and value, over 110 000 tons per year. Germany, Italy, and Belgium are processing countries.

10.4.2 Consumption of Hazelnuts Worldwide

Annual consumption of shelled hazelnuts, from 2004 to 2012, was volatile but experienced an overall decrease of 4%, expressed as a yearly average rate growth of 1.4% as shown in Table 10.9. This scenario was not worse thanks to Turkish consumption. Turkey, the main producer of hazelnuts, is also responsible for almost 25% of world consumption. Excluding Turkish hazelnut consumers, the world's consumption decreased overall by around 22%.

Table 10.9 Shelled hazelnuts consumption (tons) in the period 2004–12 (elaboration based on INC) (INC 2009, 2013).

										Variation	
	2004	2005	2006	2007	2008	2009	2010	2011	2012	Overall	Mean
Turkey	23 269	66 052	48 142	12 050	87 466	45 000	45 661	54 419	84 214	261.9%	[illegible]1.8%
Italy*	82 186	86 055	129 29	79 397	93 429	79 660	67 342	103 250	71 884	-12.5%	3.3%
Germany*	73 830	62 871	65 306	74 820	63 515	28 980	27 380	24 830	28 553	-61.3%	-8.2%
France	25 115	21 470	24 297	29 827	20 584	14 149	26 907	26 890	27 880	11.0%	6.6%
USA	18 874	12 138	18 680	17 127	17 024	17 450	11 897	14 968	15 067	-20.2%	0.8%
Russia	10 442	8959	10 967	12 991	13 866	8647	11 013	13 922	13 174	26.2%	5.5%

Canada	–	–	–	–	–	4811	7885	10 198	11 105	130.8%	34.0%
Spain	9529	5008	12 483	10 375	14 743	9640	12 089	11 270	10 922	14.6%	13.5%
Poland	1657	1 834	1518	9757	6040	6235	6946	7 023	10 159	513.1%	69.8%
Switzerland*	12 064	12 308	11 994	11 898	11 347	9767	9311	9 398	8 556	-29.1%	-4.1%
WORLD	373 280	377 022	414 074	338 446	423 593	273 501	291 599	345 234	357 993	-4.1%	1.4%

* Processing country.

World consumption per capita in 2012 was about 52 g of hazelnuts (INC 2013). When consumption data are translated into consumption per capita, the average Georgian consumed about 1.805 kg of hazelnuts in 2012, followed by Italians and Turks, with 1.188 and 1.151 kg of hazelnuts, respectively (INC 2013). The main evolution in terms of consumption between 2004 and 2012 occurred in Poland (513.1%) and Turkey (261.9%). In contrast, consumption reduced considerably in Germany (61.3%).

10.5 Walnut

Walnuts are divided into two types: Persian or English walnut (*Juglans regia* L.) and black walnut (*Juglans nigra* L.). The first type originated in Persia while the second is from North America, being more common in the USA.

Walnuts are sold in-shell after drying or shelled. The nutmeat is consumed whole, in halves, or used in pieces of different grades in several food products. Walnuts are used for the confection of snacks (chocolate bars, nougat, caramelized nut mixes, energy bars, etc.), bakery products (inclusion in bread, cookie decorations, sheet cake decorating, pastry filling, etc.), frozen dairy products for toppings, and savory products (as paste, soup, sauces, toppings, seasoned breads, and seasoning blends).

Walnuts are considered almost as a medicine with diversified

health benefits (Hayes *et al*. 2016). From a nutritional point of view, walnuts are an excellent source of fat, considered a healthy fat, because no other nut provides such amounts of polyunsaturated fatty acids (PUFA), some of them essential fatty acids (Amaral *et al*. 2003). Walnuts also provide plant protein, dietary fiber, plant sterols, and polyphenols, responsible for part of the bioactive potential exhibited by these nuts (Pereira *et al*. 2008).

Figure 10.8 demonstrates that since 2000, walnut production, harvested area, and yield have increased considerably. Production nearly doubled, while harvested area increased about 65% and yield multiplied from 2.12 tons/ha in 2000 to 3.46 tons/ha in 2013 (FAOSTAT 2015).

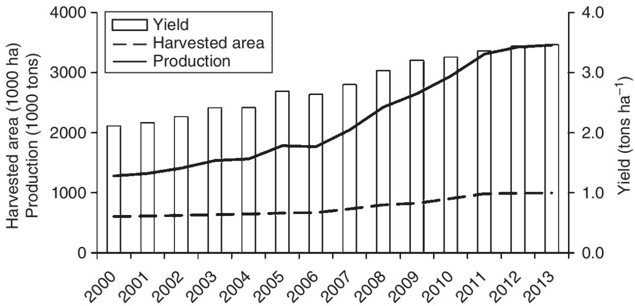

Figure 10.8 Evolution of walnut production, harvested area, and yields from 2000 to 2013 (FAOSTAT 2015).

Figure 10.9 shows the main producing countries and their contribution to walnut production.

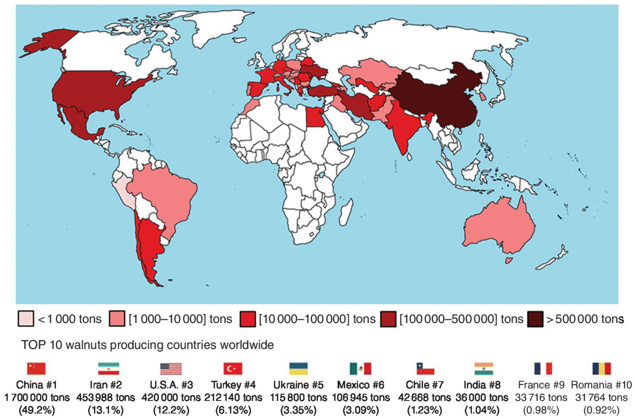

Figure 10.9 Worldwide walnut production (tons) and top 10 producers for 2013 (FAOSTAT 2015).

Overall, the top 10 producing countries account for 91% of world production. China is by far the biggest walnut producer, followed by Iran and USA with 49%, 13.1%, and 12.2% of world production, respectively. In China, according to the International Society for Horticultural Science, the most common varieties are Liaoning, Zhonglin, Xiangling, and Jinlong (ISHS 2015). In the USA several varieties are cultivated but five of them account for 80% of production: Chandler, Hartley, Howard, Payne, and Serr (UCDavis 2015).

Chinese walnut production is mainly for domestic demand, with approximately 80% of the country's production consumed domestically. Iranian walnut exports are insignificant and the country's production is intended to satisfy domestic demand. In contrast, about 40% of USA walnut production is sent to foreign markets .

10.5.1 Evolution of Walnut Production and Trade Facts

Walnut production (with shell) in 2012 reached nearly 3.43 million tons, corresponding to US$14 490 000 (Table 10.10). This volume has increased 168% since 2000, leading to an annual increase of 8.7%, on average, from the beginning of this century. This evolution is even more extraordinary in relation to production values: 620% increase since 2000. This outstanding percentage could be due to the effect of price growth of over 160%, e.g. 9.4% per year on average, in the period 2000–12. These findings demonstrate the increasing market valorization of walnuts, also verified by the change in producers' unit price, from US$1.57 per kg in 2000 to US$4.23 per kg in 2012, an increase of 169%.

Table 10.10 World production and trade of walnuts (2000–12 period) (elaboration based on FAOSTAT data; FAOSTAT 2015).

	World traded[d,e]		
	Production	With shell	Shelled

		shell									
Year	**Volume (tons)**	**Gross[a] value (US $1000)**	**Unit price[b] (US $)**	**Implicit price[c] index (2000 = 100)**	**Volume index (2000 = 100)**	**Volume (tons)**	**Value (US $1000)**	**Unit price (US $)**	**Volume (tons)**	**Value (US $1000)**	**Unit price (US $)**
2000	1 280 467	2 013 600	1.57	100.00	100.00	97 297	174 776	1.80	69 224	224 238	3.24
2001	1 323 697	2 102 260	1.59	103.51	103.38	87 071	153 711	1.77	74 461	260 584	3.50
2002	1 411 374	1 573 540	1.12	71.64	110.22	99 140	155 182	1.57	87 114	288 151	3.31
2003	1 535 902	2 010 430	1.31	85.58	119.95	99 613	173 532	1.74	94 020	336 060	3.57
2004	1 563 622	2 474 740	1.58	103.14	122.11	124 191	238 168	1.93	112 652	410 204	3.64
2005	1 783 813	3 248 920	1.82	117.29	139.31	110 126	238 983	2.17	126 528	535 646	4.23
2006	1 766 007	3 479 470	1.97	127.20	137.92	120 389	284 272	2.36	135 168	682 379	5.05
2007	2 045 746	5 250 930	2.57	160.88	159.77	123 999	317 045	2.56	141 514	805 771	5.69
2008	2 424 530	6 925 740	2.86	178.47	189.35	132 247	390 644	2.95	143 525	902 571	6.29
2009	2 648 796	8 237 030	3.11	191.22	206.86	200 788	517 618	2.58	163 132	787 861	4.83

2010	2 943 573	10 257 700	3.49	216.82	229.88	198 646	591 084	2.98	170 854	997 460	5.84
2011	3 307 729	13 914 030	4.21	262.29	258.32	217 612	793 967	3.65	169 315	1 298 315	7.67
2012	3 425 834	14 490 160	4.23	260.62	267.55	224 724	793 419	3.53	178 043	1 452 651	8.16

a Production relates to nuts in the shell or in the husk.

b Current prices, calculated without any deductions for seed.

c Price received by farmers for 1 kg of product.

d Amount related to the average of exports and imports.

e Export values are mostly reported as FOB and import values mostly as CIF.

In 2012, world trade flow of walnuts exceeded US$2 246 000, corresponding to 224 000 tons of walnuts with shell (above 6.5% of the world production) and 178 000 tons of shelled walnuts. At the beginning of the decade, international walnut trade was mostly with shell. This changed over the years and in the 2005–08 triennium the world trade volume of shelled walnuts outperformed that of walnuts with shell. In recent years, the proportion of shelled/with shell has once more favored walnuts with shell. According to statistics on the last five years from the INC (INC 2015), only approximately 35% of shelled walnut world production is directed to world trade. There is a significant difference in price between with-shell and shelled walnuts, which are, on average, more than twice the price reached by walnuts with shell. Furthermore, international trade has experienced increased appreciation of shelled walnuts; prices rose 152% in the period 2000–12, against an increase of only 97% for walnuts with shell. Table 10.11 presents the world top exporters and importers of shelled walnuts.

Table 10.11 Top world exporters and importers of walnuts (three year average) (elaboration based on FAOSTAT data; FAOSTAT 2015).

Shelled				With shell			
Exports[a]		Imports[b]		Exports[a]		Imports[b]	
Volume (tons)	**Value (US $1000)**	**Volume (tons)**	**Value (US $1000)**	**Volume (tons)**	**Value (US $1000)**	**Volume (tons)**	**Value (US $1000)**
USA 74 632	USA 576 325	Germany 15 920	Germany 136 533	USA 118 677	USA 424 455	China 50 463	China 157 945
Ukraine 25 618	Mexico 136 672	Russia 12 076	Japan 92 119	France 28 247	France 110 816	Turkey 28 949	Italy 105 418
Mexico 20 403	Chile 121 265	Japan 10 107	South Korea 82 510	China 25 124	Mexico 93 138	Italy 26 055	Turkey 76 710
Moldova 10 934	Ukraine 90 370	South Korea 9286	Spain 80 823	Mexico 22 738	Chile 64 356	Mexico 16 466	Mexico 60 084
Chile 9429	Moldova 75 692	Spain 8728	Canada 61 188	Chile 14 569	China 51 346	Spain 14 676	Spain 53 620
Others 67 076	Others 342 080	Others 155 176	Others 959 098	Others 29 906	Others 73.97	Others 51 451	Others 180 452

[a] Export values are mostly FOB.

[b] Import values are mostly reported as CIF.

The USA is the main exporter of walnuts worldwide, with around 75 000 and 119 000 tons per year of shelled walnuts and with shell, respectively. It provides about 50% of the shelled walnut world export volume and value, and 36% and 43% of walnuts with shell total exports, in volume and value, respectively. The USA's main foreign markets include South Korea, Japan, Germany, Canada, and Australia. On the other hand, imports of shelled walnuts are quite diverse and the five leading importers, Germany, Russia, Japan, South Korea, and Spain, only represent 25% of world volume imports. Moreover, import of walnuts with shell is more concentrated and the three main importers (China, Turkey, and Italy) take roughly 55% of imports.

10.5.2 Consumption of Walnuts Worldwide

World consumption of walnuts has intensified, with a rate growth

from 2004 to 2012 of 48.3% overall, with an average yearly increase of around 5% (Table 10.12).

Table 10.12 Shelled walnuts consumption (tons) in the period 2004–12 (elaboration based on INC) (INC 2009, 2013).

										Variation	
Country	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**Overall**	**Mean**
China	126 660	144 952	160 350	187 026	171 266	196 302	160 885	172 823	181 625	43.4%	5.3%
USA	107 821	101 684	105 708	87 123	123 823	119 745	161 703	128 363	144 493	34.0%	5.8%
Turkey	39 950	34 275	34 111	31 054	29 487	32 527	29 157	29 029	29 857	–25.3%	–3.3%
France	13 851	15 875	17 377	16 287	14 519	15 934	18 824	17 086	16 271	17.5%	2.6%
Japan	11 256	15 158	13 600	9 142	10 399	8 813	13 224	13 006	12 409	10.2%	4.2%
Germany	8970	10 912	10 930	11 727	8476	10 571	13 826	14 264	12 085	34.7%	5.6%
Russia	–	–	–	–	–	10 732	13 200	10 625	9134	–14.9%	–3.5%
Italy	8365	10 404	11 661	7822	9852	8813	9234	8295	9124	9.1%	2.9%
Spain	5265	8168	6399	8573	7497	10 149	8972	8952	7951	51.0%	8.4%
South Korea	4096	4931	7613	6517	6445	10 343	9262	9749	12 416	203.1%	17.7%
WORLD	341 255	378 604	406 087	393 030	421 512	435 983	436 510	509 208	506 013	48.3%	5.2%

World walnut consumption per capita increased from 50 g to 73 gg from 2004 to 2012 (INC 2009, 2013). These results are mainly from Chinese and North American consumption; together they are responsible for more than 60% of world demand and 55% of its increase. Regarding consumption per capita, the USA, Israel, and Turkey are the main consumers with 0.468, 0.438, and 0.408 kg of walnuts per capita in 2012, respectively (INC 2013). South Korea recorded the highest consumption increase between the years

2004–12 (203.1%), while Turkey reduced its consumption by 25.3% (see Table 10.12).

10.6 Conclusion

Nuts are a very important economic source for many countries worldwide. Despite often being marginal in economic productive terms in national agro-food industries, nut farms are of vital importance for the areas in which cultivation of these products is strongly rooted. Chestnut cultivation in Europe, for example, is very often carried out in mountainous areas where other sorts of farming are difficult and other economic opportunities are scarce. Historically rooted in local farming, although being an additional activity for most of the farmers, chestnut cultivation is an important source of income, ensuring not only that local communities can continue but also that the land receives the care and attention needed to prevent physical and environmental degradation.

Statistics show that the position of nuts in the international markets is increasing and will continue to increase in years to come. More recently, producers are investing to increase yields and nut quality, by applying more efficient agronomic practices and improving technology and efficiency in the orchards. Nuts are also becoming more important in the diets of many countries, which has lead to their consumption rising in recent years, a factor probably related to the many reports of their health benefits.

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11
Recent Advances in Our Knowledge of the Biological Properties of Nuts

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11.1 Introduction

Consumers tend to underestimate tree nuts as a nutritious and healthful snack; they put them into the same category as potato chips, nachos, and Cheetos. Nuts, including peanuts which are in fact a legume, are often viewed as high-fat food items with too many calories that should be eaten only sparingly to avoid weight gain. Emerging research from epidemiological studies and clinical trials is demonstrating that tree nuts, as part of a balanced diet, promote satiety and weight maintenance and in fact are not culprits of body weight gain. Moreover, tree nuts contain a plethora of nutrients and bioactive compounds (e.g. phytochemicals and phytosterols), which are now being recognized for bestowing health benefits. As will be discussed in this chapter, tree nuts have been associated with improving heart health, lowering low-density lipoprotein cholesterol levels, improving cognitive function and endothelial compliance, reducing inflammation, and even lowering cancer risks. The strongest evidence that tree nuts are cardioprotective comes from (i) epidemiological observations indicating a consistent and well-defined inverse association between the frequency of nut consumption and development of coronary heart disease, and (ii) several short-term clinical trials demonstrating the beneficial effects of nut intake on lipid profiles as well as other intermediate markers of heart disease. From the

nutrient perspective, tree nuts are a nutrient-dense food that supplies heart-healthy mono- and polyunsaturated fats, high-quality vegetable protein, dietary fiber as well as important vitamins and minerals. For example, just two Brazil nuts can provide the daily requirement of selenium, an important mineral for improving the body's antioxidant defense mechanisms.

Tree nuts are convenient, nutritious, and tasty snacks that can easily be incorporated into our busy lifestyles. They are generally eaten whole, either raw or roasted with added salt or flavorings, but also are found in confectionery and bakery products. Because of their health-promoting attributes, tree nuts have been referred to as a natural functional food. The mechanism of their actions likely is due to synergistic interactions amongst the many bioactive constituents within the nutmeat, which may favorably influence human physiology. One might say that these year-round nutritional powerhouse are truly Mother Nature's gift.

11.2 Nuts as a Source of Nutrients, Phytosterols, and Natural Antioxidants

11.2.1 Nuts as a Source of Energy and Macronutrients

Tree nuts are considered as an excellent energy source. Many efforts have been made in studying compositional information of major tree nuts. The proximate compositions of all tree nuts are summarized in Table 11.1, with triacylglycerols being the predominant component; the lipid contents in tree nuts vary from 53.5% in almonds to 75.1% in pine nuts (Miraliakbari & Shahidi 2008). This high lipid-containing nature has marked them as an excellent energy source. Nuts are generally low in available carbohydrate and glycemic index, ranging from 27.5 to 28.0 g/100 g in pistachios to 12.3 g/100 g in Brazil nuts. Tree nuts are also a rich source of protein, with the highest content found in peanuts, walnuts, almonds, pistachios, and cashews. Brazil nuts, hazelnuts, and pine nuts possess a low amount of protein with the lowest

found in pecans and macadamia nuts (Brufau *et al.* 2006).

Table 11.1 Proximate composition of tree nuts (g/100 g nutmeat, fw).

Nutrient	Almond	Brazil nut	Cashew	Chestnut	Hazelnut	Hickory nut	Macadamia	Pecan	Pine nut	Pistachio	Walnut
Moisture	3.1–9.5	3.1–3.5	4.4–8.0	45.3–52.0	4.0–5.3	2.7	1.4–2.1	3.5–7.4	1.5–2.3	3.9–5.7	2.7–4.7
Lipids	43.3–50.6	66.4–67.1	42.8–43.9	45.3–52.0	59.8–61.5	64.4	66.2–75.8	66.2–72.0	61.7–68.4	44.4–45.4	64.5–65.2
Proteins	19.5–23.3	13.9–14.3	18.2–20.9	1.6–7.4	14.1–20.6	12.7	7.9–8.4	7.5–9.2	13.1–13.7	19.8–20.6	13.5–15.2
Ash	2.5–4.6	3.3–3.5	2.5–2.8	1.0–2.9	2.0–2.3	2.0	1.1–1.2	1.5–1.9	2.5–2.6	3.0–3.2	1.8
Carbohydrates	19.7–27.0	12.3	24.1–30.2	44.2–62.3	10.0–16.7	18.3	13.8	13.9	13.1	27.5–28.0	13.7
Dietary fiber	11.8–13.0	7.5	1.4–3.3	2.3–3.7	3.4–9.7	6.4	8.6	9.6	3.7	10.3	6.7

References: Ruggeri *et al.* (1998); Venkatachalam & Sathe (2006); Çağlarırmark (2003); Çağlarırmark and Batkan (2005); De Leon and Delores (2004); USDA National Nutrient Database for Standard Reference, Release 27.

11.2.2 Biological Value of Nut Proteins

Similar to many other plants, the quality of tree nut proteins is considered to be suboptimal, as their amino acid profiles are incomplete (Table 11.2). According to the FAO/WHO, a pattern of indispensable ("essential" is an antiquated term) amino acids are recommended for children between the ages of two and five years. Tryptophan is the first limiting amino acid for a majority of tree nut proteins, with the exception being macadamias, which are limited by lysine. The predominant amino acids in tree nut proteins are aspartic and glutamic acids. For adults, on the other hand, the proteins of tree nuts contain adequate amounts of indispensable amino acids except for almonds, which are deficient in methionine and cysteine (Alasalvar & Shahidi 2009).

Table 11.2 Amino acids in tree nuts (g/100 g of portion).

Amino acid	Almond	Brazil nut	Cashew	Chestnut	Hazelnut	Macadamia	Pecan	Pine nut	Pistachio	Walnut
Tryptophan	0.211	0.135	0.287	0.027	0.193	0.067	0.093	0.107	0.271	0.170
Threonine	0.601	0.365	0.688	0.086	0.497	0.370	0.306	0.370	0.667	0.596
Isoleucine	0.751	0.518	0.789	0.095	0.545	0.314	0.336	0.542	0.893	0.625
Leucine	1.473	0.190	1.472	0.143	1.063	0.602	0.598	0.991	1.542	1.170
Lysine	0.568	0.490	0.928	0.143	0.420	0.018	0.287	0.540	1.142	0.424
Methionine	0.157	1.124	0.362	0.057	0.221	0.023	0.183	0.259	0.335	0.236
Cysteine	0.215	0.306	0.393	0.077	0.277	0.006	0.152	0.289	0.355	0.208
Phenylalanine	1.132	0.639	0.951	0.102	0.663	0.665	0.426	0.524	1.054	0.711
Tyrosine	0.450	0.416	0.508	0.067	0.362	0.511	0.215	0.509	0.412	0.406
Valine	0.855	0.760	1.094	0.135	0.701	0.363	0.411	0.687	1.230	0.753
Arginine	2.465	2.140	2.123	0.173	2.211	1.402	1.177	2.413	2.012	2.278
Histidine	0.539	0.409	0.456	0.067	0.432	0.195	0.262	0.341	0.503	0.391
Alanine	0.999	0.609	0.837	0.161	0.730	0.388	0.397	0.684	0.914	0.696
Aspartic acid	2.639	1.325	1.795	0.417	1.679	1.099	0.929	1.303	1.803	1.829
Glutamic acid	6.206	3.190	4.506	0.312	3.710	2.267	1.829	2.926	3.790	2.816
Glycine	1.429	0.733	0.937	0.124	0.724	0.454	0.453	0.691	0.946	0.816
Proline	0.969	0.706	0.812	0.127	0.561	0.468	0.363	0.673	0.805	0.706
Serine	0.912	0.676	1.079	0.121	0.735	0.419	0.474	0.835	1.216	0.934

Reference: USDA National Nutrient Database for Standard Reference, Release 27.

Most tree nut proteins are rich in arginine, ranging from 2.47 g/100 g fresh weight (fw) nutmeat in almonds to 1.40 g/100 g fw nutmeat in macadamias, while the lowest arginine content of 0.173 g/100 g fw nutmeat was reported for chestnuts. Arginine can be metabolized to nitric oxide (NO), an important signaling molecule and a potent vasodilator, by endothelial NO synthase (Förstermann & Sessa 2012). Nut proteins generally have a lower lysine/arginine ratio than proteins from animal sources. This ratio is reportedly associated with a significantly lower risk of developing hypercholesterolemia and atherosclerosis, which also decreases the risk of cardiovascular diseases (Brufau *et al.* 2006).

11.2.3 Nuts as a Source of Vitamins and Minerals

Tree nuts are plant-based powerhouses packed with a combination of macronutrients, vitamins, and minerals. Studies have shown that tree nuts are rich in tocopherols (vitamin E), which is not surprising because of their high lipid values (Miraliakbari & Shahidi 2008). Vitamin contents of major nut types are summarized in Table 11.3. Four tocopherol isomers were reported in all tree nuts at various levels, while tocotrienols (data not shown) were found to a much lesser extent (Robbins *et al.* 2011). The predominant tocopherol homologue in tree nut oils is γ-tocopherol, with the exception being almond and hazelnut lipids, which are high in α-tocopherol. The levels of both the α- and γ-isomers are similar in pine nut oil. α-Tocopherol is widely considered as the most bioactive homologue because of its high affinity to the tocopherol transfer protein in the liver. However, the exceptional property of γ-tocopherol is receiving much attention. Research has indicated that γ-CEHC, the metabolite of γ-tocopherol, might have an antiinflammatory effect as demonstrated by its downregulating capacity of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) (Jiang & Ames 2003; Jiang *et al.* 2000, 2001).

Table 11.3 Vitamins in tree nuts (fw).

Vitamin	Almond	Brazil nut	Cashew	Chestnut	Hazelnut	Macadamia	Pecan	Pine nut	Pistachio	Walnut
Vitamin	0.0	0.7	0.5	43.0	6.3	1.2	1.1	0.8	5.6	1.3
C	0.205	0.617	0.423	0.238	0.643	1.195	0.660	0.364	0.870	0.341
(mg/100	1.138	0.035	0.058	0.168	0.113	0.162	0.130	0.227	0.160	0.150
g)	3.618	0.295	1.062	1.179	1.800	2.473	1.167	4.387	1.300	1.125
Thiamine	0.471	0.184	0.864	0.509	0.918	0.758	0.863	0.313	0.520	0.570
(mg/100	0.137	0.101	0.417	0.376	0.563	0.275	0.210	0.094	1.700	0.537
g)	44	22	25	62	113	11	22	34	51	98
Riboflavin	2	–	–	28	20	–	56	29	415	20
(mg/100	25.63	5.65	0.90	–	15.03	0.54	1.40	9.33	2.30	0.70
g)	0.23	0.01	0.03	–	0.33	–	0.39	0.00	0.00	0.15
Niacin	0.64	9.56	5.31	–	0.00	–	24.44	11.15	22.60	20.83

(mg/100 g)	0.07 –	0.63 –	0.36 34.1	– –	0.00 14.2	– –	0.47 3.5	0.00 53.9	0.80 –	1.89 2.7
Pantothenic acid (mg/100 g)										
Vitamin B_6 (mg/100 g)										
Folate, total (mg/100 g)										
Vitamin A (IU/100 g)										
α-Tocopherol (mg/100 g)										
β-Tocopherol (mg/100 g)										
γ-Tocopherol (mg/100 g)										
δ-Tocopherol (mg/100 g)										
Vitamin K (μg/100										

g)

Reference: USDA National Nutrient Database for Standard Reference, Release 27.

Regarding mineral content (Table 11.4), in general, tree nuts are rich in magnesium, manganese, phosphorus, and potassium. Almonds and cashews are recognized as an excellent nondairy source of calcium and iron, respectively. It is important to mention that Brazil nuts have a considerably higher content of selenium than any other tree nut type. Selenium intake is strongly related to the redox status in the human body. While selenium itself does not act as an antioxidant directly, it functions as a catalyst for glutathione peroxidase, an important component in the endogenous antioxidant defense system in the human body (Battin & Brumaghim 2009).

Table 11.4 Mineral content in tree nuts portion (fw).

Compound	Almond	Brazil nut	Cashew	Chestnut	Hazelnut	Macadamia	Pecan	Pine nut	Pistachio	Walnut
Calcium, Ca (mg/100 g)	269	160	37	27	114	85	70	16	105	98
Iron, Fe (mg/100 g)	3.71	2.43	6.68	1.01	4.70	3.69	2.53	5.53	3.92	2.91
Magnesium, Mg (mg/100 g)	270	376	292	32	163	130	121	252	121	158
Phosphorus, P (mg/100 g)	481	725	593	93	290	188	277	575	490	346
Potassium, K (mg/100	733	659	660	518	680	368	410	597	1025	441
	1	3	12	3	0	5	0	2	1	2
	3.12	4.06	5.78	0.52	2.45	1.30	4.53	6.45	2.20	3.09
	1.031	1.743	2.195	0.447	1.725	0.756	1.200	1.324	1.300	1.586
	2.179	1.223	1.655	0.952	6.175	4.131	4.500	8.802	1.200	3.414
	4.1	1917.0	19.9	–	2.4	3.6	3.8	0.7	7.0	4.9

g)
Sodium, Na (mg/100 g)
Zinc, Zn (mg/100 g)
Copper. Cu (mg/100 g)
Manganese, Mn (mg/100 g)
Selenium, Se (µg/100 g)

Reference: USDA National Nutrient Database for Standard Reference, Release 27.

11.2.4 Nuts as a Source of Essential Fatty Acids and Phytosterols

Many studies have been carried out on tree nut fatty acids and minor lipid constituents. Although rich in lipid content, the beneficial action of tree nut consumption on maintaining body weight and glucose homeostasis has been validated by clinical trials and intervention studies conducted over the past decade or so (García-Lorda *et al.* 2003; Griel & Kris-Etherton, 2006; King *et al.* 2008; Mattes *et al.* 2008; Schwingshackl & Hoffmann 2012). The fact may seem paradoxical but the healthful fatty acid profiles of tree nuts are responsible for these protective effects. The fatty acid compositions of common tree nuts are summarized in Table

11.5. The lipids of tree nuts are generally high in unsaturated fats, with the exception of Brazil and cashew nut oils. Although tree nut oils differed considerably in their levels of individual fatty acids, oleic acid (C18:1 ω-9) and linoleic acid (C18:2 ω-6) are considered as the two predominant ones. The oleic acid (O) and linoleic acid (L) ratio (O/L) is an important factor related to the quality and stability of oil products. The O/L ratio is greatest in hazelnuts, while the lowest ratios were reported for pine nut and walnut oils. Of particular note is that walnuts are the only tree nut containing a significant amount of α-linolenic acid (C18:3 ω-3).

Table 11.5 Fatty acid composition of tree nuts (g/100 g oil).

Nut type	**C16:0**	**C18:0**	**C20:0**	**C16:1 ω7**	**C18:1 ω9**	**C20:1 ω9**	**C18:2 ω6**	**C18:3 ω3**
Almond	6.00–6.45	1.47–2.10	–	0.40–0.43	65.70–67.62	–	24.03–24.80	Trace
Brazil nut	12.63–14.71	9.79–11.63	–	0.29	29.76–38.36	–	36.84–45.17	0.074
Cashew	10.31–11.14	9.08–9.83	0.68–0.74	0.34	56.87–60.57	0.19	17.03–22.22	0.21
Hazelnut	5.02–5.78	1.89–2.36	0.12–0.18	0.16–0.19	79.57–79.64	0.15–0.16	11.78–12.72	0.08
Macadamia	8.04–8.78	2.34–3.74	1.96–2.88	17.95–20.8	54.1–60.08	2.62–2.53	2.32–3.74	–
Pecan	6.15	2.54	–	–	62.36	–	27.69	1.25
Pine nut	4.08–5.22	2.36–2.78	0.41–0.42	0.08	24.82–27.67	1.32–1.38	45.02–46.41	19.28
Pistachio	9.17–11.79	1.23–1.5	–	1.07	55.11–56.75	–	28.56–29.45	0.33–0.37
Walnut	6.00–7.11	2.00–2.72	0.07	0.07	14.80–16.96	0.19	58.64–63.10	11.67–13.43

References: Robbins *et al.* (2011); Zadernowski *et al.* (2009); Chandrasekara *et al.* (2011); Amaral *et al.* (2006); Crews *et al.* (2005a and 2005b). C16:0, palmitic acid; C18:0, stearic acid; C20:0 arachidic acid; C16:1 ω7, palmitoleic acid; C18:1 ω9, oleic acid; C20:1 ω9, gondoic acid; C18:2 ω6, linoleic acid; C18:3 ω3, α-linolenic acid.

Analysis of the unsaponifiables indicated that β-sitosterol is the

most abundant sterol in all tree nut types, followed by stigmasterol and campesterol. Please see Table 11.6 for phytosterol distributions in tree nuts. Pistachio oil contains a significantly higher quantity of β-sitosterol (260.5 mg/100 g of nut). Intake of rich amounts of phytosterols in tree nut oils can eventually lead to a reduction of serum low-density lipoprotein (LDL) and total cholesterol levels (Sathe *et al.* 2009).

Table 11.6 Phytosterol composition of tree nuts (mg/100 g oil).

Nut type/ processing	Campesterol	Stigmasterol	β-Sitosterol	Δ5-Avenasterol	Δ7-Avenasterol	Δ5,24-Stigmastadienol
Almond	5.50–10.58	5.17–6.59	207.2–322.2	43.91	–	9.24
Brazil nut	1.75	8.02	91.40	32.07	–	–
Cashew	19.37	1.57	250.0	16.89	–	5.65
Hazelnut	10.09–11.36	1.85–2.43	156.0–202.6	6.16–12.23	0.98	2.30
Macadamia	7.33–12.32	3.83	150.7–196.5	20.75	–	2.54
Pecan	8.20	3.28	167.0	10.00	–	–
Pine nut	32.07	2.75	197.8	66.18	–	6.64
Pistachio	21.68	3.82	441.0	44.58	–	6.32
Walnut	7.76–8.28	0.60–1.34	142.5–153.9	8.60–13.73	1.45	3.32

References: Robbins *et al.* (2011); Crews *et al.* (2005a,b); Maguire *et al.* (2004); Ryan *et al.* (2006).

11.2.5 Phenolic Compounds Originating from Tree Nuts as Natural Antioxidants

Tree nuts are a rich source of phenolic compounds (Tables 11.7 and 11.8). Their antioxidant properties have been confirmed in numerous studies using varying experimental systems (Table 11.9).

Table 11.7 Content of total phenolics in tree nuts.

Nuts	Total phenolics	Reference
Almond	239	Kornsteiner *et al.* 2006; values are expressed as mg gallic acid equivalents/100 g of nuts, fw (mg GAE/100 g)
Brazil nut	112	
Cashew	137	
Hazelnut	291	–
Macadamia	46	–
Peanut	420	–
Pecan	1284	–
Pine nut	32	–
Pistachio	867	–
Walnut	1625	–
Almond (Crude extract)	16.1 ± 0.4	Amarowicz *et al.* 2005; values are expressed as mg (+)-catechin equivalents/g of crude extract (mg CE/g)
Almond (LMW fraction)	7.14 ± 0.2	
Almond (HMW fraction)	80.4 ± 2.1	–
Almond	4.18 ± 0.84	Wu *et al.* 2004; values are expressed as mg gallic acid equivalents/g of nuts, fw (mg GAE/g)

Brazil nut	3.10 ± 0.96	
Cashew	2.74 ± 0.39	–
Hazelnut	8.35 ± 2.16	–
Macadamia	1.56 ± 0.29	–
Peanut	3.96 ± 0.54	–
Pecan	20.16 ± 1.03	–
Pine nut	0.68 ± 0.25	–
Pistachio	16.57 ± 1.21	–
Walnut	15.56 ± 4.06	–

HMW, high molecular weight; LMW, low molecular weight.

Table 11.8 Content of flavonoids according to USDA database (mg/100 g edible portion (f w)).[a]

Nuts	Anthocyanidins	Catechins	Flavanones	Flavonols
Almonds	2.43 (0.00–4.40)[b]	3.91 (1.97–4.25)	0.68 (0.03–1.62)	3.03 (1.02–11.03)
Brazil nuts	–	–	–	–
Cashew	–	1.98 (0.00–3.82)	–	–
Hazelnuts	6.71 (4.40–13.60)	5.93 (0.00–7.23)	–	–
Macadamias	–	–	–	–
Pecans	7.29 (4.47–11.07)	15.99 (4.89–25.83)	–	–
Pine nuts	–	0.49 (0.00–0.75)	–	–
Pistachios	7.33 (3.15–14.30)	6.85 (2.62–18.07)	–	1.56 (0.00–4.30)
Walnuts	2.71 (2.11–3.74)	–	–	–

The numbers in brackets are min/max values reported in the database.

a USDA Database for the Flavonoid Content of Selected Foods: http://www.ars.usda.gov/News/docs.htm?docid=6231.

b Flavonoids content are listed for their mean value, minimum and maximum values reported in the database.

Table 11.9 Antioxidant capacity of tree nuts.

Nuts	Method	Activity	Unit	Reference
Yellow cashew	ABTS	3.322	mmol TE/100 g dw	Moo-Huchin *et al.* 2015
	ABTS	0.970	mg vit. C/100 g dw	
	DPPH	1.579	mmol TE/100 g dw	–
	DPPH	0.340	mg vit. C/100 g dw	–
Red cashew	ABTS	3.050	mmol TE/100 g dw	–
	ABTS	0.890	mg vit. C/100 g dw	–
	DPPH	1.593	mmol TE/100 g dw	–
	DPPH	0.343	mg vit. C/100 g dw	–
Almond (Crude extract)	TAA	0.24 ± 0.02	µmol Trolox/mg extract	Amarowicz *et al.* 2005
Almond (LMW fraction)	TAA	0.09 ± 0.01	µmol Trolox/mg extract	–
Almond (HMW fraction)	TAA	3.93 ± 0.31	µmol Trolox/mg extract	–
Almond	H-ORAC$_{FL}$	42.82 ± 8.71	µmol of TE/g fw	Wu *et al.* 2004
Brazil nut	H-ORAC$_{FL}$	8.62 ± 2.06	µmol of TE/g fw	–
Cashew	H-ORAC$_{FL}$	15.23 ± 2.04	µmol of TE/g fw	–
Hazelnut	H-ORAC$_{FL}$	92.75 ±	µmol of TE/	–

		17.78	g fw	
Macadamia	H-ORAC$_{FL}$	14.43 ± 2.31	µmol of TE/g fw	
Peanut	H-ORAC$_{FL}$	28.93 ± 2.36	µmol of TE/g fw	
Pecan	H-ORAC$_{FL}$	175.24 ± 10.36	µmol of TE/g fw	
Pine nut	H-ORAC$_{FL}$	4.43 ± 1.11	µmol of TE/g fw	
Pistachio	H-ORAC$_{FL}$	75.57 ± 10.50	µmol of TE/g fw	
Walnut	H-ORAC$_{FL}$	130.57 ± 35.20	µmol of TE/g fw	

ABTS - 2,2′-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid; DPPH - 2,2′-diphenyl-1-picrylhydrazyl radical; dw, dry weight; fw, fresh weight; ORAC, oxygen radical absorbance capacity; TE, trolox equivalents.

Extracts prepared from whole almond seeds and their brown skins showed antioxidant activity evaluated using a cooked comminuted pork model, a β-carotene-linoleate model, and a bulk stripped-corn oil system. In the cooked comminuted pork model system, brown skin extracts inhibited the formation of 2-thiobarbituric acid reactive substances (TBARS), total volatiles, and hexanal more effectively than the whole seed extract. RP-HPLC analysis revealed the presence of caffeic, ferulic, *p*-coumaric, and sinapic acids as the major phenolic acids in the extracts examined (Wijeratne *et al.* 2006). Phenolic compounds present in a crude extract of almonds and its fractions, after separation on a lipophilic Sephadex LH-20 column, showed antioxidant and antiradical properties, as revealed following studies using a β-carotene-linoleate model system, the total antioxidant activity method, DPPH radical-scavenging assay, and reducing power evaluation. Results of these assays showed the highest values of antioxidant activity for the tannin fraction. Another RP-HPLC analysis of a crude extract from almond seeds revealed the presence of vanillic, caffeic, *p*-coumaric, and ferulic acids (after basic hydrolysis), as well as quercetin, kaempferol, and isorhamnetin (after acidic hydrolysis), delphinidin and cyanidin (after *n*-butanol-HCl hydrolysis), and procyanidin B_2 and B_3 (Amarowicz *et al.* 2005).

Monagas *et al*. (2009) reported strong antioxidant capacity of roasted skins obtained from the industrial processing of peanuts, hazelnuts, and almonds as well as fractions containing low and high molecular weight bioactives. The total antioxidant capacity, $ORAC_{FL}$, DPPH radical-scavenging test, and reducing power assays were employed in this study. Roasted peanut and hazelnut skins presented similar total phenolics contents, much higher than that of almond skins; yet their flavan-3-ol profiles, as determined by LC-ESI-MS and MALDI-TOF-MS, differed considerably. Peanut skins were low in monomeric flavan-3-ols (19%) in comparison to hazelnut (90%) and almond (89%) skins.

The phenolic compounds of crude extracts of hazelnut skin and their low molecular weight and tannin constituents exhibited strong antiradical activity against the ABTS radical cation and DPPH radical, as well as reducing power. These results suggest that hazelnut skins can be considered as a value-added by-product for use as dietary antioxidants (Alasalvar *et al*. 2009). The antioxidant activity of a crude hazelnut extract and its fractions was confirmed by the ABTS radical cation and DPPH radical-scavenging assays, reducing power, and β-carotene-linoleate model system. In the extract, five phenolic acids, namely gallic, caffeic, *p*-coumaric, ferulic, and sinapic acids, were tentatively identified and quantified, among which gallic acid was the most abundant in both free and esterified forms (Alasalvar *et al*. 2006).

Anderson *et al*. (2001) observed that copper-mediated LDL oxidation was inhibited by 84% in the presence of a walnut extract. During the same study, plasma TBARS formation was significantly inhibited by the walnut extracts. Almond-pellicle flavonoids increased the resistance of copper-mediated LDL oxidation *in vitro* and *ex vitro* and acted synergistically with vitamins C and E (Chen *et al*. 2005).

Consumption of hazelnuts (1 g/day/kg body weight) improved oxidative stress markers (e.g. malon(di)aldehyde levels in plasma and plasma antioxidant capacity) in human studies (Durak *et al*. 1999). In other human clinical trials, the consumption of walnuts, almonds, and almond oil did not affect LDL’s oxidizability (Hyson *et al*. 2002; Iwamoto *et al*. 2002).

Phenolic extracts from defatted pecan nutmeat have demonstrated strong antioxidant capacity against reactive radical species *in vitro*. Hudthagosol *et al.* (2011) conducted a randomized, placebo-controlled, cross-over trial with pecan consumption. Results showed increased γ-tocopherol and proanthocyanidin (PAC) postprandial levels after pecan consumption. This study indicated that the bioactive constituents from pecans are absorbable and contribute to the postprandial antioxidant defenses in the human body. Robbins *et al.* (2014, 2015) investigated the antioxidant capacity of pecan phenolic extracts using *in vitro* methods, and compositional information of low and high molecular weight pecan fractions by RP-HPLC-MS^n after their separation from a crude acetonic extract via a Sephadex LH-20 column. The mass spectral results showed ellagic acid, its derivatives, and proanthocyanidins, mostly of two and three degrees of polymerization, to be prominent contributors.

11.3 Health Benefits of Nuts

11.3.1 Health-Promoting Properties of Nuts

It is now widely accepted that a healthy diet plays a vital role in reducing the risk of disease and achieving optimal health and development. A number of epidemiological studies and clinical trials in recent decades have revealed an inverse relationship between nut consumption and chronic diseases. The outcomes of these researches have led to a move towards issuing a health claim for nut products. Tree nuts are a unique package of healthful fats, plant protein, minerals, and vitamins. The combination of these beneficial nutrients is most likely responsible for their proposed health benefits. O'Neil *et al.* (2015) conducted a survey assessing the nutrient adequacy of tree nut consumers based on the National Health and Nutrition Examination data from 2005 to 2010. The results showed that tree nut consumers comprised a lower

percentage (p <0.0001) of the population below the estimated average requirement (EAR) for vitamins A, E, and C, folate, calcium, iron, magnesium, and zinc and thus possessed better nutrient adequacy than nonconsumers.

11.3.2 Nuts and Body Weight Control

It is common for consumers to believe that frequent consumption of high-fat foods like nuts could have an antagonist effect on body weight maintenance and glucose homeostasis. However, epidemiological studies do not support this concern. Excellent reviews regarding the effect of nut intake on body weight control have been published by García-Lorda *et al.* (2003), Sabaté (2003), Vadivel *et al.* (2012), Jackson & Hu (2014), and Tan *et al.* (2014). These review articles point out that nut incorporation into the diet is unlikely to promote weight gain, despite an expected increase of total caloric intake. On the contrary, regular nut consumption may aid in maintaining body weight balance. More detailed research on the bioactive constituents and long-term feeding trials featuring tree nuts are nevertheless warranted to substantiate these purported findings.

11.3.3 Nuts and Cardiovascular Disease

Many studies have consistently suggested that frequent nut consumption might protect against and reduce the risk of coronary heart disease (CHD) and cardiovascular disease (CVD) by improving serum blood lipids (Kris-Etherton 2014; Sabaté & Wien 2013). Some results from clinical investigations are summarized in Table 11.10. For instance, Hu and Stampfer (1999) estimated that substitution of fat from 0.0283 kg of nuts for equivalent energy from carbohydrate in an average diet was associated with a 30% reduction in CHD risk, and the substitution of nut fat for saturated fat was associated with 45% reduction in risk.

Table 11.10 Nut consumption and cardiovascular-related diseases.

Nuts	Conclusions	Reference

Baru almond (*Dipteryx alata* Vog.)	Dietary supplementation of mildly hypercholesterolemic subjects with baru almonds improved serum lipid parameters, so that this food might be included in diets for reducing the CVD risk	Bento *et al.* 2014
Brazil nuts	Brazil nuts intake improved the lipid profile and microvascular function in obese adolescents, possibly due to their high level of unsaturated fatty acids and bioactive substances	Maranhão *et al.* 2011
Macadamia nuts	Macadamia nuts can be included in a heart-healthy dietary pattern that reduces lipid/ lipoprotein CVD risk factors	Griel *et al.* 2008
Peanut	Regular peanut consumption lowers serum TAG, augments consumption of nutrients associated with reduced CVD	Alper & Mattes 2003

	risk, and increases serum magnesium concentration	
Peanut	The results suggest that frequent nut and peanut butter consumption is associated with a significantly lower CVD risk in women with type 2 diabetes	Li *et al.* 2009
Pistachio	Inclusion of pistachios in a healthy diet beneficially affects CVD risk factors in a dose-dependent manner, which may reflect effects on plasma stearoyl-CoA desaturase activity (SCD)	Gebauer *et al.* 2008
Pistachio	A significant decrease in small and dense LDL (sdLDL) levels was observed following the two and one serving of pistachios per day. The inclusion of pistachios in a moderate-fat diet favorably affects the cardiometabolic profile in individuals with an	Hooligan *et al.* 2014

	increased risk of CVD	
Walnut	The restructured meat products with added walnuts supplied in this study can be considered functional foods for subjects with high risk for CVD, as their regular consumption provokes a reduction in total cholesterol of 4.5% with respect to baseline values (mixed diet) and 3% with respect to the restructured meat without walnuts	Olmedilla-Alonso *et al.* 2008
Walnut	In experiments with rats, a diet containing walnuts prevented hyperleptinemia and decreased the total cholesterol compared with the control	Domínguez-Avila *et al.* 2015
Walnut	The results suggest that the walnut extract has a high antiatherogenic potential and a remarkable	Papoutsi *et al.* 2008

	osteoblastic activity, an effect mediated, at least in part, by its major component ellagic acid. Such findings suggest the beneficial effect of a walnut-enriched diet on cardioprotection	
Walnut	Flow-mediated vasodilation (FMD) of the brachial artery improved significantly from baseline when subjects consumed a walnut-enriched diet as compared with the control diet	Katz *et al.* 2012
Tree nuts	Two ounces of nuts daily as a replacement for carbohydrate foods improved serum lipids in type 2 diabetes	Jenkins *et al.* 2011
Tree nuts	The consumption of nuts is associated with a marked decrease in CVD risk in large population-based studies. Nut consumption is also associated with clinically relevant	Good *et al.* 2009

	reduction in LDL cholesterol without adversely affecting HDL cholesterol or causing a significant amount of weight gain	
Tree nuts	Prospective studies in non-Mediterranean populations have consistently related increasing nut consumption to lower coronary heart disease mortality	Guasch-Ferré *et al.* 2013
Tree nuts	An increased risk of stroke was observed among participants who never consumed nuts compared with those consuming nuts	Di Giuseppe *et al.* 2015
Tree nuts	Substitution of the carbohydrate and saturated fatty acids in an average diet with 28.35 g of nuts of equivalent energy was associated with a reduction in CHD	Hu & Stampfer 1999
Tree nuts	Because of their unique nutrient profile, nuts can be part of a diet that	Griel & Kris-Etherton 2006

	features multiple heart-healthy foods resulting in a cholesterol-lowering response that surpasses that of cholesterol-lowering diets typically used to reduce CVD risk	
Tree nuts	Nut consumption is associated with clinically relevant reduction in LDL cholesterol (-9% to -16%) without adversely affecting HDL cholesterol or causing a significant amount of weight gain	Good *et al.* 2009

CHD, coronary heart disease; CVD, cardiovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TAG, triglyceride.

The results of research conducted by Fraser *et al.* (1992) strongly suggest that frequent consumption of nuts may protect against the risk of CHD events. The favorable fatty acid profile of many nuts is one possible explanation for such an effect. Subjects who consumed nuts frequently (e.g. more than four times per week) experienced substantially fewer fatal CHD events and definite nonfatal myocardial infarctions, compared to those who consumed nuts less than once a week.

In the etiology and development of atherosclerosis, plaque plays an important role in chronic inflammation. Activation of the vascular endothelium is an early inflammatory event in the development of atherosclerosis leading to endothelium dysfunction

and its consequences (Hansson 2005). Nuts contain several active compounds/bioactives that exhibit antiinflammatory activity; these include ω-3 polyunsaturated fatty acids (PUFA), dietary fiber, magnesium, L-arginine, and some antioxidants (Salas-Salvadó *et al.* 2008).

Jiang *et al.* (2006) examined associations between nut and seed consumption and C-reactive protein (CRP), interleukin-6, and fibrinogen in the Multi-Ethnic Study of Atherosclerosis. This 2000 cross-sectional analysis included 6080 USA participants aged 45 and 84 years old with adequate information on diet and biomarkers. The authors concluded that frequent nut and seed consumption was associated with lower levels of inflammatory markers.

In the study by Salas-Salvadó *et al.* (2008) with a total of 339 men and 433 women aged between 55 and 80 years at high cardiovascular risk, the consumption of some typical Mediterranean foods (e.g. fruits, cereals, virgin olive oil, and nuts) was associated with lower serum concentrations of inflammatory markers, especially those related to endothelial function. Subjects with the highest consumption of nuts showed the lowest concentrations of vascular cell adhesion molecule (VCAM-1) and intracellular adhesion molecule (ICAM-1), IL-6, and CRP.

Hshieh *et al.* (2015) conducted a prospective cohort study with 20 742 male physicians. The study investigated nut intake between 1999 and 2002 via a food-frequency questionnaire and ascertained deaths through an endpoint committee. The results substantiated the inverse association between nut consumption and the risk of all-cause and cardiovascular disease mortality amongst all subjects.

Compared to a placebo, supplementation of the diet of 20 mildly hypercholesterolemic subjects (total cholesterol = 5.8 ± 0.2 mmol/L) in a randomized, cross-over, placebo-controlled study with 20 g/day of baru almonds (*Dipteryxalata* Vog.; a native species of almond from Brazil) reduced total cholesterol by 8.1%, LDL cholesterol by 9.4%, and non-high-density lipoprotein (HDL) cholesterol by 8.1% (Bento *et al.* 2014). The improvement of the serum fatty acid profile was found to be dose dependent.

In a randomized, cross-over clinical trial conducted by Nishi *et al.* (2014), 27 healthy hyperlipidemic subjects completed three one-month dietary phases featuring two almond (full and half) and a control phase. Each phase was separated by a washout period lasting a minimum of two weeks. The study revealed that almond consumption favorably altered the serum fatty acids by increasing their total monounsaturated fatty acid (MUFA) content, most notably oleic acid. These changes in the fatty acid profile were postulated as being associated with a lower CHD risk.

In a randomized, placebo-controlled clinical trial, the consumption of Brazil nuts improved the lipid profile and microvascular function in obese female adolescents (n = 17), possibly due to their high level of unsaturated fatty acids and bioactive substances (Maranhão *et al.* 2011).

Twenty-one hypercholesterolemic adults participated in a double-control sandwich model intervention with a single group and three isoenergetic diet periods for a total of 12 weeks (Orem *et al.* 2013). The findings indicated that a hazelnut-enriched diet significantly improved flow-mediated dilation (FMD) by 56.6%. Oxidized LDL, high sensitivity CRP, and soluble VCAM-1 levels from the group ingesting the hazelnut diet were significantly lower compared to those of the control diet group. It was suggested that regular consumption of a hazelnut-enriched diet can improve endothelial function and prevent oxidation of LDL. An improvement in the status of these biomarkers is thought to be responsible for the cardioprotective effects.

In a study with 6309 women with type 2 diabetes, frequent consumption of peanuts and peanut butter (i.e. five servings per week; 26 g nuts per serving and 16 g butter per serving) was inversely associated with total CVD risk in age-adjusted analyses. Increased nut consumption was significantly associated with a more favorable plasma lipid profile, including lower LDL cholesterol, non-HDL cholesterol, total cholesterol, and apolipoprotein-B-100 concentrations (Li *et al.* 2009).

In the study conducted by Alper and Mattes (2003), 15 normolipidemic adults participated in a 30-week cross-over intervention; the subjects were provided 500 kcal as peanuts during an eight-week free feeding (FF) diet. The same quantity of

peanuts was added during a three-week addition (ADD) diet or replaced an equal amount of other fats in the diet during an eight-week substitution (SUB) diet. Serum triacylglycerol concentrations were reduced by 24% during ADD, by 17% during SUB, and by 14% during four weeks of free feeding. In conclusion, regular peanut consumption was shown to lower serum triacylglycerols, augment consumption of nutrients associated with reduced CVD risk, and increase serum magnesium concentrations.

From a randomized, cross-over, controlled feeding study with 28 subjects, a significant decrease in small and dense LDL (sdLDL) levels was observed after one and two servings of pistachios per day (comprising 30% and 34% of total fat). Furthermore, reductions in sdLDL levels were correlated with reduced TAG levels following the two servings versus the control group (Holligan *et al.* 2014). In a similar study involving 28 individuals with LDL cholesterol levels greater than or equal to 2.86 mmol/L, two servings/day of a pistachio diet (20% energy from pistachios) resulted in decreased total cholesterol (-8%), LDL cholesterol (-11.6%), non-HDL cholesterol (-11%), apo B (-4%), apo B/apo A-I (-4%), and plasma of stearoyl-CoA desaturase activity (SCD) (Gebauer *et al.* 2008).

In experiments by Domínguez-Avila *et al.* (2015), a diet containing whole pecans prevented hyperleptinemia and decreased the content of total cholesterol in blood compared to that of the control. The high fat in the whole pecans (HF + WP) diet upregulated the hepatic expression of apolipoprotein B and LDL receptor mRNAs with respect to the high fat levels. Addition of pecan oil to the diet resulted in a reduced level of triacylglycerols in the blood compared with that of the control.

Regular consumption of walnut-enriched meat products for five weeks compared with restructured meat products devoid of added walnuts resulted in a decrease in total cholesterol by 12.8% in test subjects. Compared to baseline (mixed diet) data, meat products with walnuts decreased total cholesterol (17.1%), LDL cholesterol (13%), and increased γ-tocopherol (16.8%) levels (Olmedilla-Alonso *et al.* 2008). Daily ingestion of 56 g of walnuts improved endothelial function in overweight adults with visceral adiposity

(Katz *et al.* 2012).

As the endothelial cell expression of adhesion molecules has been recognized as an early step in atherogenesis, Papoutsi *et al.* (2008) examined the effect of a methanolic extract of walnuts as well as ellagic acid, one of the walnut's major polyphenolic components, on the expression of vascular cell adhesion molecule (VCAM-1) and intracellular adhesion molecule (ICAM-1) in human aortic endothelial cells. The walnut extract and ellagic acid significantly decreased the tumor necrosis factor (TNF)-α-induced endothelial expression of both VCAM-1 and ICAM-1. The acquired results suggest that the walnut extract possesses a high antiatherogenic potential.

11.3.4 Nuts and Diabetes

The contents of MUFAs, PUFAs, dietary fiber, vegetable proteins, and polyphenols play an essential role in reducing risk factors for diabetes complications. Some of the findings to date are summarized in Table 11.11.

Table 11.11 Nut consumption and type 2 diabetes.

Nuts	Conclusions	Reference
Almonds	For 22 postmenopausal women with type 2 diabetes, a diet with the addition of almonds has clinically beneficial effects on lipid- and lipoprotein-mediated CVD risk	Richmond *et al.* 2013
Hazelnuts	Incorporation of hazelnuts into the diet can prevent reduction of HDL-C concentrations in	Damavandi *et al.* 2013

	patients with type 2 diabetes	
Tree nuts	Pooled analyses show a metabolic syndrome (MetS) benefit of tree nuts through modest decreases in fasting blood glucose	Mejia *et al.* 2014
Tree nuts	Pooled analyses show that tree nuts improve glycemic control in individuals with type 2 diabetes, supporting their inclusion in a healthy diet	Viguiliouk *et al.* 2014
Tree nuts	Consuming 56.7 g of nuts daily as a replacement for carbohydrate foods improved both glycemic control and serum lipids in type 2 diabetes	Jenkins *et al.* 2011
Tree nuts	In two large, independent cohorts of nurses and other health professionals, the frequency of nut consumption was inversely associated with total and cause-specific mortality, independently of	Bao *et al.* 2013a

	other predictors of death	
Walnut	A walnut-enriched *ad libitum* diet improves endothelium-dependent vasodilation in type 2 diabetic individuals, suggesting a potential reduction in overall cardiac risk	Ma *et al.* 2010
Walnut	The consumption of walnuts was inversely associated with risk of type 2 diabetes, and the associations were largely explained by Body Mass Index (BMI). The results suggest that higher walnut consumption is associated with a significantly lower risk of type 2 diabetes in women	Pan *et al.* 2013
Walnut	Walnut methanolic extract showed a strong α-glucosidase inhibitory activity with IC_{50} values of 80 μg/mL	Sancheti *et al.* 2011

CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol.

Viguiliouk *et al.* (2014) conducted a systematic review and meta-analysis of randomized-controlled trials to assess the effects of tree nuts on glycemic markers in individuals with diabetes. Pooled analyses show that tree nuts improve glycemic control in subjects with type 2 diabetes. Review articles published by Kendall *et al.* (2010a,b) suggested that nut consumption had minimum effects on rising postprandial blood glucose levels; instead, it can suppress the rise in blood glucose levels when consumed with other carbohydrate-dense foods. Fasting blood glucose, as an effect of tree nut consumption, was also underlined by Mejia *et al.* (2014). Unfortunately, the number of clinical trials on the onset and prevalence of type 2 diabetes and tree nut consumption is limited.

In the Jenkins *et al.* (2011) study, a total of 117 type 2 diabetic subjects were randomized to one of three treatments for three months. Supplements were provided at 475 kcal per 2000 kcal diet as mixed nuts (75 g/day), muffins, or half portions of both. Improved glycemic control and serum lipids in type 2 diabetes subjects were observed with 0.0567 kg of nuts daily as a replacement for carbohydrate foods.

The association between nut consumption and the risk of type 2 diabetes was studied in a prospective cohort of 20 224 male participants (Kochar *et al.* 2010). While nut consumption was associated with a lower risk of type 2 diabetes in a model adjusted for age, this relation was attenuated upon additional controls for other confounders from the lowest to the highest category of nut consumption, respectively.

Twenty-two postmenopausal women with type 2 diabetes consumed personalized diets, with the addition of 30 g/day of almonds. All food was supplied for two periods of three weeks, separated by a four-week washout. The findings revealed that total and LDL cholesterol decreased significantly (Richmond *et al.* 2013).

In an eight-week controlled, randomized parallel study of patients with type 2 diabetes, 50 eligible volunteers were assigned to either the control or intervention groups. The replacement of 10% of the

total daily caloric intake with hazelnuts in the intervention group had no effect on fasting blood sugar levels (Damavandi *et al.* 2013).

The results of Pan *et al.* (2013) suggest a beneficial effect of walnut consumption. In the multivariable-adjusted Cox proportional hazards model without body mass index (BMI), walnut consumption was associated with a lower risk of type 2 diabetes, and the HRs for participants consuming 1–3 servings/month (1 serving = 0.028 kg), 1 serving/week, and ≥ 2 servings/week of walnuts were compared with women who never or rarely consumed walnuts.

According to Ma *et al.* (2010), a walnut-enriched diet improves endothelium-dependent vasodilation in type 2 diabetic individuals, suggesting a potential reduction in overall cardiac risk. This study was a randomized, controlled, single-blind, cross-over trial. Twenty-four participants with type 2 diabetes (mean age 58 years; 14 women and 10 men) were randomly assigned to one of two possible sequence permutations: to receive an *ad libitum* diet enriched with 0.056 kg (366 kcal) walnuts/day or an *ad libitum* diet without walnuts for eight weeks. A walnut extract also demonstrated strong α-glucosidase inhibitory activity with IC_{50} value of 80 μg/mL (Sancheti *et al.* 2011).

Nuts can be served as a specific food option for diabetic patients to reduce carbohydrate intake. There is enough evidence to suggest that incorporating nuts, including peanuts, into daily diets can protect against type 2 diabetes and other metabolic syndromes associated with diabetes, even though the exact mechanisms are still unknown. However, the beneficial actions were likely attributed to the reduction in oxidative damage and inflammatory biomarkers in blood lipids. Further efforts are warranted on detailed phytochemical compositions and more interventional studies to elucidate the mechanism regarding the preventive potential of nuts.

11.3.5 Nuts and Cancer

Macronutrients, micronutrients, and bioactive compounds (e.g.

phenolics, phytoestrogens) present in nuts can act in the prevention of cancer (Falasca *et al.* 2014; Papanastasopoulos & Stebbing 2013). Some studies of this potential are reported in Table 11.12.

Table 11.12 Nut consumption and cancer.

Nuts	Conclusion	Reference
Fermented almonds, macadamias, hazelnuts, pistachios, and walnuts	This study presents the chemopreventive effects (reduction of tumor-promoting deoxycholic acid, rise in chemopreventive short chain fatty acids, protection against oxidative stress) of different nuts after *in vitro* digestion and fermentation, and shows the potential importance of nuts in the prevention of colon cancer	Lux *et al.* 2012
Tree nuts	Recent studies have suggested that nut consumption is associated with reduced cancer mortality	Falasca *et al.* 2014
Tree nuts	There are numerous mechanisms of action by which the biological-active compounds of nuts can intervene in the	Falasca & Casari 2012

	prevention of cancer, although they have not been fully elucidated	
Tree nuts	New epidemiological studies are required to clarify the possible effects of nuts on cancer, particularly prospective studies that make reliable and complete estimations of their consumption and which make it possible to analyze their effects independently of the consumption of legumes and seeds	González & Salas-Salvadó 2006
Tree nuts	Nuts consumed during adolescence were associated with reduced breast cancer risk	Liu *et al.* 2014
Tree nuts	Frequent nut consumption is inversely associated with risk of pancreatic cancer in this large prospective cohort of women, independent of other potential risk	Bao *et al.* 2013b

	factors for pancreatic cancer	
Tree nuts	These findings support the hypothesis that dietary intake of fiber and nuts during adolescence influences subsequent risk of breast disease and may suggest a viable means for breast cancer prevention	Su *et al.* 2010
Walnuts	The results support an effect of walnut and its bioactive constituents on mammary epithelial cells and that multiple molecular targets may be involved	Vanden Heuvel *et al.* 2012
Walnuts	Walnuts in the diet inhibit colorectal cancer growth by suppressing angiogenesis	Nagel *et al.* 2012
Walnuts	Walnut phenolic extracts showed concentration-dependent growth inhibition toward human kidney and colon cancer cells. The results strongly	Carvalho *et al.* 2010

indicate that walnuts constitute an excellent source of effective natural antioxidants and chemopreventive agents

There are numerous supposed mechanisms of this action: scavenging of free radicals, regulation of differentiation, inhibition of chemical-induced carcinogenesis, regulation of DNA damage, regulation of inflammatory response and immunological activity, induction of phase 2 metabolic enzymes, and regulation of hormone mechanisms (González & Salas-Salvadó 2006). The number of epidemiological studies is limited. Sabaté and Ang (2009) emphasized that studies in the past two decades have examined only three tumor sites, and the benefits appear to be manifested only in women. Several authors concluded that further research is necessary (González & Salas-Salvadó 2006; Jenab *et al.* 2004; Nagel *et al.* 2012; Sabaté & Ang 2009). One of the greatest difficulties in interpreting the results of such studies is that the consumption of nuts, legumes, and seeds is often investigated and reported together (González & Salas-Salvadó 2006).

According to Falasca *et al.* (2014), recent studies have suggested that nut consumption is associated with reduced cancer mortality. This evidence reinforces the interest in investigating the chemopreventive properties of nuts, and it raises questions about the specific cancer type(s) and settings that may be affected by nut consumption, as well as the cellular mechanisms involved in this protective effect.

The results of the European Prospective Investigation into Cancer and Nutrition study – a large prospective cohort study involving 10 European countries – showed no association between higher intake of nuts and seeds and the risk of colorectal, colon, and rectal cancers in men and women combined; however, a significant inverse association was observed in subgroup analyses for colon cancer in women at the highest (>6.2 g/day) versus the lowest category of intake and for the linear effect of log-transformed intake, with no associations in men (Jenab *et al.* 2004).

Grosso *et al.* (2015) conducted a systematic review and meta-analysis of perspective studies that explored the effects of nut consumption on CVD and cancer mortality. The results showed that nut consumption was tightly associated with lower risk of cancer mortality when comparing highest and lowest nut intake categories although the author suggested that no dose effect was observed.

Inverse associations were found between intakes of dietary fiber, vegetable protein, vegetable fat, and nuts during adolescence and breast cancer risk, which persisted after controlling adult intakes (Liu *et al.* 2014).

Bao *et al*. (2013b) prospectively followed 75 680 women in the Nurses' Health Study and examined the association between nut consumption and pancreatic cancer risk. Frequent nut consumption was inversely associated with risk of pancreatic cancer, independent of other potential risk factors for pancreatic cancer.

The findings of Su *et al*. (2010) support the hypothesis that dietary intake of fiber and nuts during adolescence influences subsequent risk of breast disease and may suggest a viable means for breast cancer prevention. Women consuming greater than or equal to two servings of nuts/week had a 36% lower risk than women consuming less than one serving/month.

The results from the pilot study of Jia *et al*. (2006) indicate that almond consumption has preventive effects on oxidative stress and DNA damage caused by smoking. The levels of two known biomarkers for DNA damage, urinary 8-hydroxy-2′-deoxyguanosine (8-OH-dG) and single-strand DNA breaks of peripheral blood lymphocytes, were measured by ELISA and Comet assays, respectively. The results showed lower levels of urinary 8-OH-dG and single-strand DNA breaks in the two almond-treated groups (84 g and 168 g of almonds each day, respectively for four weeks) compared with the control group.

The results of Vanden Heuvel *et al.* (2012) supported an effect of walnut and its bioactive constituents (α-linolenic acid (ALA) and β-sitosterol) on mammary epithelial cells. Lipids from walnuts decreased the proliferation of MCF-7 cells, as did ALA and β-sitosterol. An extract of walnut oil increased activity of the

farnesoid X receptor (FXR) in the mouse breast cancer cell line TM2H.

Chemopreventive effects (reduction of tumor-promoting deoxycholic acid, rise in chemopreventive short chain fatty acids (SCFA), protection against oxidative stress) of different nuts after *in vitro* digestion and fermentation, and their potential importance in the prevention of colon cancer were reported by Lux *et al.* (2012). Bioactive compounds from *in vitro* fermented almonds, macadamias, hazelnuts, and walnuts significantly reduced the growth of HT29 cells. DNA damage induced by H_2O_2 was significantly reduced by the compounds of fermented walnuts after 15 minutes co-incubation of HT29 cells.

In the research study of Nagel *et al*. (2012), HT29 human colon cancer cells were injected into six-week-old female nude mice. The growth rate of tumors was slower in walnut-fed compared to corn oil-fed animals by 27%. Walnuts and their oil significantly reduced serum expression levels of angiogenesis factors, including vascular endothelial growth factor (by 30%), and approximately doubled total necrotic areas despite smaller tumor sizes.

The results obtained by Davis and Iwahashi (2001) suggest that almond consumption may reduce the risk of colon cancer. Six-week-old male rats were fed either whole almond-, almond meal-, almond oil-containing or control diets, and were then given subcutaneous injections of azoxymethane twice one week apart. After 26 weeks the animals were injected with bromodeoxyuridine, after which colons were evaluated for aberrant crypt foci (ACF) and cell turnover (labeling index, LI). Whole-almond ACF and LI were both significantly lower than the wheat bran and cellulose diet groups (–30 and –40%, respectively), while almond meal and almond oil ACF and almond meal LI declines were only significant versus cellulose.

Dietary walnut intake suppressed mammary gland tumorigenesis in mice (Hardman *et al*. 2011), when compared to a diet without walnuts. Consumption of walnuts significantly reduced tumor incidence (fraction of mice with at least one tumor), multiplicity (number of glands with tumor/mouse), and size. Gene expression analyses indicated that consumption of the walnut diet altered expression of multiple genes associated with the proliferation and

differentiation of mammary epithelial cells.

Hardman and Ion (2008) performed a pilot study to determine whether consumption of walnuts could affect growth of MDA-MB 231 human breast cancers implanted into nude mice. Tumor cells were injected into nude mice that were consuming a control diet with 10% corn oil. After the tumors reached a 3–5 mm diameter, the diet of one group of mice was changed to include ground walnuts, equivalent to 56 g per day in humans. The tumor growth rate from day 10, when tumor sizes began to diverge, until the end of the study was significantly less for the group that consumed walnuts than the group that did not. Tumor cell proliferation decreased, but apoptosis was not altered as a result of walnut consumption.

Reiter *et al.* (2013) investigated whether a standard mouse diet supplemented with walnuts reduced the establishment and growth of LNCaP human prostate cancer cells. The walnut-enriched diet reduced the number of tumors and the growth of the LNCaP xenografts. Similarly, the xenografts in the walnut-fed animals grew more slowly than those in the control diet mice. The final average tumor size in the walnut-diet animals was roughly one-fourth the average size of the prostate tumors in the mice that ate the control diet.

Aligning with the beneficial effects of nut consumption on cardio- and diabetes-related diseases, scientific evidence has suggested an association between nut intake and cancer risk reduction. However, the types of cancer examined were limited, and the nuts were usually grouped with other seeds and legumes. Future research, in particular large prospective cohort studies, is needed to substantiate and reinforce the rationale of the effects of nut consumption on cancer risk.

11.3.6 Application of Nuts in the Functional Food Industry

Nutrient-dense tree nuts and peanuts are an excellent addition to savory snacks for boosting energy or assuaging hunger. Food manufacturers have launched flavored tree nuts and peanut snacks along with their unflavored counterparts. The diversification of

product lines can greatly increase consumer acceptance of nut snacks loaded with potential health beneficial properties. A novel spin-off, such as polyphenol-rich peanut skin-fortified peanut butter, has been formulated and investigated (Ma *et al.* 2013). The proanthocyanidin-rich red skin of peanuts is a major waste product from peanut-processing plants. Incorporation of the skins up to 3.75% into peanut butters significantly increased the total phenolics content (TPC) compared with nonfortified counterparts, with minimum perceivable changes in physical texture of the product.

A high lipid-bearing nature and heart-healthy lipid profile have made tree nuts good candidates for specialty oils. Although the size of this niche market is relatively small, it has expanded remarkably over the last decade, as more health-conscious consumers are demanding healthier alternatives with better taste. Tree nuts can be prepared for oil expression after adequate cleaning and shelling. The oil can either be mechanically pressed or extracted with a food-grade solvent. Mechanically pressed oils are bottled directly after filtration and nitrogen flush to prevent oxidation. These premium-priced products are commonly used in salad dressings or as dipping sauces to bring signature nutty flavors.

11.4 Tree Nuts and Allergy

Several authors reported nuts as one of the main food groups causing allergic reactions (Crespo *et al.* 2006; Roux *et al.* 2003; Sathe *et al.* 2009). The concerns about peanut and tree nut allergy have increased along with their growing popularity in recent years, as the nutritional benefits of nut consumption have become more widely known. It is estimated that approximately 1% of the USA population is allergic to peanuts and/or tree nuts. Most of the peanut and tree nut allergens are caused by their storage proteins, which are resistant to heat treatment and complete digestion in the gastrointestinal (GI) tract.

Because of the prevalence of peanut allergies, their allergenicities are the most studied cases. Experimental investigations have

shown that the majority of peanut allergens are resistant to digestion *in vitro* (Fu *et al.* 2002; Sathe *et al.* 2005). IgE immunoreactivity of purified Ara h 1 and Ara h 2 prepared from roasted peanuts was higher than that of their counterparts prepared from raw and boiled peanuts. The IgE-binding capacity of purified Ara h 1 and Ara h 2 was altered by heat treatment, and in particular was increased by roasting. However, no significant difference in IgE immunoreactivity was observed between whole protein extracts from raw and roasted peanut products. The decrease in allergenicity of boiled peanuts results mainly from a transfer of low molecular weight allergens into the water during cooking (Mondoulet *et al.* 2005).

In the experiment of Schmitt *et al*. (2010), the soluble and insoluble fractions of peanuts that were boiled, fried, and roasted were subjected to electrophoresis and Western blot analysis using anti-Ara h 1 and anti-Ara h 2 antibodies and serum IgE from peanut-allergic individuals. Overall, protein solubility is reduced with processing and IgE binding increases in the insoluble fractions, due mostly to the increase in the amount of insoluble proteins with increased time of heating in all processes tested. Therefore, it can be concluded that thermal processing of peanuts alters solubility, and the differences in protein solubility within various extract preparations may contribute to inconsistent skinprick test and immunoassay results, particularly when nonstandardized reagents are used.

The protein fractions of peanuts were altered to a similar degree by frying or boiling. Compared with roasted peanuts, the relative quantity of Ara h 1 was reduced in the fried and boiled preparations, resulting in a significant reduction of IgE-binding intensity. In addition, there was significantly less IgE binding to Ara h 2 and Ara h 3 in fried and boiled peanuts compared with that in roasted peanuts, even though the protein amounts were similar in all three preparations (Beyer *et al*. 2001).

The findings of Chung and Champagne (2007) revealed that phytic acid formed complexes with the major peanut allergens (Ara h 1 and Ara h 2), which were insoluble in acidic and neutral conditions. Succinylation of the allergens inhibited complex formation, indicating that lysine residues were involved. A six-fold

reduction in IgE binding or allergenic potency of the peanut protein extract was observed after treatment with phytic acid. It was concluded that phytic acid formed insoluble complexes with the major peanut allergens, and resulted in a reduced allergenic potency. Application of phytic acid to a peanut butter slurry presented a similar result, indicating that phytic acid may find use in the development of hypoallergenic peanut-based products.

The findings of Chung *et al*. (2004) indicate that peroxidase can help reduce roasted peanut allergens. In their experiments, protein extracts from raw and roasted defatted peanut meals at pH 8 were incubated with and without peroxidase in the presence of H_2O_2. Results showed that peroxidase treatment had no effect on raw peanuts with respect to protein cross-linking. However, a significant decrease was evident in the levels of the major allergens, Ara h 1 and Ara h 2, in roasted peanuts after peroxidase treatment; moreover, polymers were formed. Despite this, a reduction in IgE binding was observed. It was concluded that peroxidase induced the cross-linking of mainly Ara h 1 and Ara h 2 from roasted peanuts and that, due to peroxidase treatment, IgE binding was reduced.

Pomés *et al*. (2006) studied the effect of roasting on Ara h 1 quantification in peanuts by using a specific monoclonal antibody-based ELISA. Ara h 1 levels were up to 22-fold higher in roasted than in raw peanuts. Inhibition ELISA tests indicated that this increase was not due to conformational changes in the Ara h 1 monoclonal antibody epitopes; rather, these results suggest that roasting increases the efficiency of Ara h 1 extraction, and/or that the monoclonal antibody binding epitopes were more accessible in roasted peanuts.

The stability of amadin (14S legumine-like protein of almonds) to thermal processes (e.g. blanching, roasting, and autoclaving) was confirmed by Roux *et al*. (2001). The authors used Western blots and almond-allergic human serum. In the experiments of Venkatachalam *et al*. (2002), only prolonged roasting and microwave heating for three minutes significantly decreased the allergenic properties of almonds. In this investigation, antigenicity was measured with antialmond rabbit polyclonal antibodies, not human IgE.

Incidence of allergy after tree nut consumption covered almost all types of tree nuts, but current research efforts have only been extended to almonds, Brazil nuts, cashews, hazelnuts, and walnuts. Future work in identifying allergens of other tree nuts is ongoing. To date, a total number of 32 tree nut proteins showing IgE reactivity have been isolated.

Two types of allergens, Ana o 1 and 2, have previously been identified in the extractable protein of cashew nuts, which are defined as vicilin and anacardein, respectively (Wang *et al.* 2002, 2003). It is important to point out that greater than 50% of cashew-allergic patients are reported to react with vicilin, even though it accounts for only 5% of the extractable proteins from cashew nuts.

Allergy cases stemming from Brazil nuts are less common in the USA than in the UK. A methionine-rich 2S albumin protein, Ber e 1, has been identified as being responsible for the allergy reaction (Koppelman *et al.* 2005; Murtagh *et al.* 2003).

The hazelnut allergens are characterized by two reaction processes. Some patients are clinically allergic to hazelnuts via a tree pollen-sensitizing mechanism. A hazelnut allergen, Cor a 1, was reportedly responsible for this type of allergy. However, the nonpollen-related allergy to hazelnuts has not been well investigated. It is speculated that an 11S globulin, named Cor a 9, might be the cause for hazelnut allergic patients in the USA. Heat treatment at 100 °C for up to 90 minutes had no influence on the allergenicity of hazelnut proteins. The IgE binding activity of the main hazelnut allergens decreased after a 15-minute heat treatment at temperatures between 100 °C and 185 °C, and was no longer detectable at 170 °C (Wigotzkia *et al.* 2000). A boiling treatment was reported to be able to decrease the allergenic potency of chestnut (Lee *et al.* 2005).

Several proteins isolated from walnuts have been confirmed as important allergens. While most of the allergic patients reacted with rJug r 1, a recombinant 2S albumin precursor, other allergens including Jug r 2, 3, 4, and Ara h 1 are also responsible for allergic symptoms.

Pecan antigens are also stable towards digestion. Multiple IgE-reactive polypeptide bands isolated from pecan protein extracts

have shown reactivity in pecan-allergic patients (Venkatachalam *et al*. 2006). Sharma *et al*. (2011a,b) identified pecan 2S albumin, Car I I, Car I 4, and its isomers as major pecan allergens. However, human digestive enzymes were able to decrease the allergenic potency of chestnuts (Lee *et al*. 2005).

11.5 Conclusion

Tree nuts are rich in macronutrients, micronutrients, and bioactives. It is suggested that consuming nuts regularly as part of the diet is associated with better nutrient adequacy and quality. Nut lipids are generally low in saturated fats and high in mono- and polyunsaturated fats. The combination of healthful lipid constituents and the beneficial action of nut polyphenols is protective against the development of chronic diseases and cancers. Nuts can be formulated into nutritious snacks or used as food ingredients to serve as both an energy source and bioactive antioxidants; these will provide additional benefits to consumers.

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12
Nuts as Sources of Nutrients

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As an introductory note, it should be highlighted that the chemical composition of nuts depends greatly on genotypic and environmental factors, such as growing region, cultivation methods, climatic conditions, harvesting years, and kernel ripeness (Muhammad *et al*. 2015; Yada *et al*. 2011). Therefore, some of the indicated values for typical parameters are characterized by having a wide variation range, due to the great variability of results among research groups.

12.1 *Prunus dulcis* (Miller) D. A. Webb (almond)

12.1.1 Botanical Aspects and Geographical Distribution

Almond, which belongs to the genus *Prunus* and the subgenus *Amygdalus* (Rosaceae), is a nutritionally important crop grown in many temperate and subtropical regions in the world. The cultivated almond is designated as *Prunus dulcis* (Miller) D. A. Webb, despite being also known as *Prunus amygdalus* Batsch and *Prunus communis* L., or less commonly as *Amygdalus communis* L. (USDA 2010).

Almond is one of the oldest cultivated nut trees in the world and a major nut tree crop in hot arid countries of the Mediterranean basin, although it spreads around the area included between the 36th and 45th parallels (Nanos *et al*. 2002).

12.1.2 Main Applications and Nutritional Overview

Almond seeds are classified as nuts, and are widely used, especially for direct consumption after toasting and for the confectionery industry and the production of sweets, cakes, and sugar-coated almonds (Nanos *et al*. 2002). Almonds are eaten raw, roasted, and fried but they can also be used as ingredients in products such as sauces and snacks and marzipan and almond crunch. More recently, almonds are also being processed to make nutritional products such as almond milk used as a substitute for cow's milk (Aranceta *et al*. 2006). In each case, the chemical composition is of great importance to establish nutritive value and quality to satisfy the concerns of consumers wanting a healthy lifestyle. The quality of nuts is defined in particular by moisture level, lipid content, oil composition, and oil ultraviolet absorption coefficients (Nanos *et al*. 2002).

Almonds are one of the most popular nut crops, being significant for human nutrition and health. These nuts are a nutrient-rich source of lipids, protein, dietary fiber, minerals, vitamins, and polyphenols. The high nutritive value of almond kernels arises mainly from their high lipid content, which constitutes an important caloric source, without contributing to cholesterol formation in humans. Almond oil has been reported to be very rich in monounsaturated fatty acids, especially oleic acid, whereas saturated fatty acids are very low (Kodad *et al*. 2014).

Considering studies performed in Portuguese (Barreira *et al*. 2012a), American (Ahrens *et al*. 2005; Chen *et al*. 2006; López-Ortiz *et al*. 2008; Martín-Carratalá *et al*. 1998; Venkatachalam & Sathe 2006), Irish (Maguire *et al*. 2004), Spanish (Ahrens *et al*. 2005; Cherif *et al*. 2009; Piscopo *et al*. 2010), Italian (Ahrens *et al*. 2005; Martín-Carratalá *et al*. 1998; Piscopo *et al*. 2010), French (Ahrens *et al*. 2005; Piscopo *et al*. 2010), Australian

(Ahrens *et al*. 2005) and Tunisian (Ahrens *et al*. 2005; Martín-Carratalá *et al*. 1998) samples, almonds are characterized by high amounts of fat (42–57%), protein (19–23%), carbohydrates (20–27%), and fiber (11–15%), and low amounts of moisture (3–9%) and ash (2.5–4.5%). Almonds are also acknowledged for their minerals, vitamins, and polyphenols (Yada *et al*. 2011).

12.1.3 Major Components

The relevant compounds studied in almond are detailed in Table 12.1, as well as the specific methodologies applied in each case.

Table 12.1 Compounds (ordered alphabetically) analyzed in each of the nuts studied in this chapter.

Source	**Compounds**	**Analysis method**	**Reference**
Almond	Dietary fiber	Gravimetry	Yada *et al*. 2013
	Ellagitannins; gallotannins	HPLC-DAD/FD	Xie *et al*. 2012
	Fatty acids	GC-FID	Askin *et al*. 2007; Barreira *et al*. 2012a; Cherif *et al*. 2009; García-López *et al*. 1996; Kodad *et al*. 2014; Madawala *et al*. 2012; Maguire *et al*. 2004; Martín-Carratalá *et al*. 1998; Matthäus & Özcan 2009; Piscopo *et al*.

		2010; Soler *et al*. 1988; Venkatachalam & Sathe 2006; Zhu *et al*. 2015
	GC-MS	Di Stefano *et al*. 2014
Minerals	ICP-AES	Prats-Moya *et al*. 1997; Yada *et al*. 2013
	AAS	Saura-Calixto & Cañellas 1982; Piscopo *et al*. 2010
Phenolic compounds	LC-DAD/FD MALDI-TOF-MS	Bartolomé *et al*. 2010 –
	HPLC-FD/MS	Xie *et al*. 2012
Proteins	Kjeldahl digestion	Ahrens *et al*. 2005; Askin *et al*. 2007; Kumar & Sharma 2005; Sathe 1993; Venkatachalam & Sathe 2006
Sterols	HPLC-DAD	Maguire *et al*. 2004
	GC-FID	Cherif *et al*. 2009; Yada *et al*. 2013
	GC-MS	Madawala *et al*. 2012
Stilbenes	UHPLC-MS	Xie & Bolling 2014
Sugars	Enzymatic kit	Amrein *et al*. 2005

	Spectrophotometry	Venkatachalam & Sathe 2006
	HPLC-RI	Balta *et al.* 2009; Barreira *et al.* 2010; Kazantzis *et al.* 2003
Tocopherols	HPLC-DAD/FD	Barreira *et al.* 2012a; Kodad *et al.* 2011, 2014; López-Ortiz *et al.* 2008; Maguire *et al.* 2004; Matthäus & Özcan 2009; Zhu *et al.* 2015
	RP-HPLC-UV	Kornsteiner *et al.* 2006
	RP-HPLC-FD	Di Stefano *et al.* 2014
Triacylglycerols	HPLC-ELSD	Barreira *et al.* 2012a
	HPLC-RI	Martín-Carratalá *et al.* 1999; Prats-Moya *et al.* 1999
Vitamin B complex	Microbiological assay	Daoud *et al.* 1977; Hoppner *et al.* 1994; Yada *et al.* 2013
	HPLC	Rizzolo *et al.* 1991
	Fluorometry	Yada *et al.* 2013

		RP-HPLC-UV	Vasconcelos *et al.* 2007
Chestnut	Ascorbic acid	HPLC-UV	Pereira-Lorenzo *et al.* 2005
	Carotenoids	HPLC-DAD	Pereira-Lorenzo *et al.* 2005
	Fatty acids	GC-FID	Barreira *et al.* 2009b, 2012b; Borges *et al.* 2007; Fernandes *et al.* 2011
	Dietary fiber	Gravimetry	Barreira *et al.* 2009b, 2012b; Gonçalves *et al.* 2010; Vasconcelos *et al.* 2007
	Minerals	AAS	Borges *et al.* 2008
		ICP-AES	Pereira-Lorenzo *et al.* 2005
	Organic acids	UFLC-PDA	Carocho *et al.* 2013
		Spectrophotometry	Gonçalves *et al.* 2010
		HPLC-UV	Ribeiro *et al.* 2007
	Phenolic compounds	RP-HPLC-UV	Gonçalves *et al.* 2010; Vasconcelos *et al.* 2007
	Protein	Kjeldahl digestion	Barreira *et al.* 2009b, 2012b;

			Gonçalves *et al*. 2010
	Starch	SEM-LFD	Cruz *et al*. 2013
		Spectrophotometry	Correia *et al*. 2012; Demiate *et al*. 2001; Vasconcelos *et al*. 2007
	Sugars	PA	Pereira-Lorenzo *et al*. 2005
		HPLC-RI	Barreira *et al*. 2010; Fernandes *et al*. 2011
		HPLC-ELSD	Bernárdez *et al*. 2004
	Tocopherols	HPLC-FD	Fernandes *et al*. 2011; Pereira-Lorenzo *et al*. 2005
		HPLC-DAD/FD	Barreira *et al*. 2009a,b, 2012b
	Triacylglycerols	HPLC-ELSD	Barreira *et al*. 2009b, 2012b, 2013
Hazelnut	Carotenoids	HPLC-UV	Kornsteiner *et al*. 2006
	Fatty acids	GC-FID	Bignami *et al*. 2005; Botta *et al*. 1994; Ciemniewska-Żytkiewicz *et al*. 2015;

			Köksal *et al*. 2006; Madawala *et al*. 2012; Oliveira *et al*. 2008; Parcerisa *et al*. 1998; Seyhan *et al*. 2007; Venkatachalam & Sathe 2006
	GLC-FID	Amaral *et al*. 2006a; Parcerisa *et al*. 1998	
Minerals	AAS		Açkurt *et al*. 1999; Alasalvar *et al*. 2009; Köksal *et al*. 2006; Seyhan *et al*. 2007
Organic acids	HPLC-UV		Botta *et al*. 1994
Phenolic compounds	HPLC-DAD-MS		Jakopic *et al*. 2011; Slatnar *et al*. 2014
	RP-HPLC-DAD-MS	Ciemniewska-Żytkiewicz *et al*. 2015	
	HPLC-PDA	Shahidi *et al*. 2007	
Proteins	Kjeldahl digestion		Köksal *et al*. 2006
Sterols	GLC-FID		Alasalvar *et al*. 2009; Amaral *et al*. 2006a;

			Parcerisa *et al*. 1998
		GC-MS	Ciemniewska-Żytkiewicz *et al*. 2015; Madawala *et al*. 2012
	Sugars	Spectrophotometry	Venkatachalam & Sathe 2006
	Tocopherols	GLC-FID	Parcerisa *et al*. 1998
		RP-HPLC-UV	Ciemniewska-Żytkiewicz *et al*. 2015; Kornsteiner *et al*. 2006
		HPLC-PDA	Alasalvar *et al*. 2009; Bignami *et al*. 2005; Köksal *et al*. 2006
	Triacylglycerols	HPLC-ELSD	Alasalvar *et al*. 2009; Amaral *et al*. 2006b
	Vitamin B complex	Microbiological assay	Açkurt *et al*. 1999
		HPLC-PDA	Köksal *et al*. 2006
Walnut	Carotenoids	HPLC-DAD	Abdallah *et al*. 2015
	Fatty acids	GC-FID	Amaral *et al*. 2003; Bouabdallah *et al*. 2014; Li *et al*. 2007; Madawala *et al*. 2012;

		Rabrenovic *et al*. 2008; Tapia *et al*. 2013; Verardo *et al*. 2009
Minerals	AAS	Lavedrine *et al*. 2000; Tapia *et al*. 2013
Phenolic compounds	HPLC-DAD	Colaric *et al*. 2005
	UHPLC-DAD-MS	Slatnar *et al*. 2015
	HPLC-DAD/LTQ-MS	Regueiro *et al*. 2014
	HSCCC-ESI-IT-TOF-MS	Grace *et al*. 2014
	NMR; ESI-MS	Zhang *et al*. 2009
	CE-ESI-TOF-MS	Gómez-Caravaca *et al*. 2008
	MEKC	Verardo *et al*. 2009
Proteins	SDS-PAGE	Labuckas *et al*. 2014
Sterols	GLC-FID	Abdallah *et al*. 2015; Amaral *et al*. 2003; Schwartz *et al*. 2008
	GC-MS	Madawala *et al*. 2012; Verardo *et al*. 2009, 2013
Tocopherols	HPLC-FD	Abdallah *et al*. 2015; Madawala *et*

		al. 2012; Schwartz *et al*. 2008; Verardo *et al*. 2013
	HPLC/UV-DAD/MS	Miraliakbari & Shahidi 2008
	HPLC-UV	Kornsteiner *et al*. 2006
	HPLC-PDA	Li *et al*. 2007
	GC-FID	Verardo *et al*. 2009, 2013
Triacylglycerols	GC-FID	Bouabdallah *et al*. 2014
Volatile compounds	GC-MS	Abdallah *et al*. 2015

AAS, atomic absorption spectrometry; AES, atomic emission spectrometry; CE, capillary electrophoresis; DAD, diode array detector; ELSD, evaporative light scattering detector; ESI, electrospray ionization; FD, fluorescence detector; FID, flame ionization detector; GC, gas chromatography; HPLC, high-performance liquid chromatography; HSCCC, high-speed counter-current chromatography; ICP, inductively coupled plasma; IEC, ionic exchange chromatography; IT, ion trap; LC, liquid chromatography; LFD, large field detector; LTQ, linear ion trap; MALDI, matrix-assisted laser desorption/ionization; MEKC, micellar electrokinetic chromatography; MS, mass spectrometry; NMR, nuclear magnetic resonance; PA, pulse amperometry; PAGE, polyacrylamide gel electrophoresis; PDA, photodiode array detection; RI, refractive index; RP, reverse phase; SDS, sodium dodecylsulfate; SEM, scanning electron microscopy; TOF, time of flight; UFLC, ultra-fast liquid chromatography; UHPLC, ultra-high performance liquid chromatography; UV, ultraviolet.

The most abundant fatty acids in almond oil are oleic acid (50.41–81.20%), linoleic acid (6.21–37.13%), palmitic acid (5.46–15.78%), stearic acid (0.80–3.83%), and palmitoleic acid (0.23–2.52%). Linolenic acid and myristic acid were also detected (Askin *et al*. 2007; Barreira *et al*. 2012a; Madawala *et al*. 2012; Zhu *et al*. 2015). The percentages of fatty acids are also reflected by the triacylglycerol profile, where OOO (30–55%) and OLO (16–3%) were the major molecules, followed by OLL (6–15%), OOP (5–13%), LOP (3–11%), SOO (0.4–4.0%), PLP (0.1–3.2%),

LLP (0.4–2.8%), and POP (0.03–0.46%), (L, linoleoyl; O, oleoyl; P, palmitoyl; S, stearoyl) (Barreira *et al*. 2012a; Martín-Carratalá *et al*. 1999; Prats-Moya *et al*. 1999).

Regarding the protein content, which is usually calculated from the amount of total nitrogen by applying specific nitrogen-to-protein conversion factors, the dominant protein in almonds is a globulin named amandin, which contains 19.3% nitrogen, corresponding to a conversion factor of 5.18. The general factor of 6.25 used in protein calculations is based on a 16% nitrogen content of many common proteins; however, using this factor for almonds would overestimate the protein content (Yada *et al*. 2011). Concerning the amino acids profile, asparagine (Asn) is by far the most abundant in almond proteins (Amrein *et al*. 2005).

Among the carbohydrates present in almond, sugars, starch, and some sugar alcohols are the only ones that can be digested, absorbed, and metabolized by humans. Nevertheless, the nonstarch fraction might promote physiological effects that are beneficial for human health. These indigestible polysaccharides (e.g. cellulose, hemicelluloses, oligosaccharides, pectins, gums, waxes) are the main components of the well-known dietary fiber (Yada *et al*. 2011). Sucrose was reported as the predominant sugar in almonds, ranging from 11.5 to 22.2 g/100 g dry weight (dw); other individual sugars were detected in minor concentrations: raffinose (0.71–2.11 g/100 g dw), glucose (0.42–1.30 g/100 g dw), maltose (0.29–1.30 g/100 g dw), and fructose (0.11–0.59 g/100 g dw) (Balta *et al*. 2009; Barreira *et al*. 2010).

12.1.4 Minor Components

Regarding the vitamins present in almond, most literature is focused on the content of tocopherols in the kernels. Vitamin E (α-, β-, γ-, δ-tocopherol and α-, β-, γ-, δ-tocotrienol) is only produced in plants, and is a strong antioxidant with a protective role in biological systems, besides having hypocholesterolemic, anticancer, and neuroprotective properties (Sen *et al*. 2007). Almonds are considered one of the richest food sources of α-

tocopherol (Barreira *et al*. 2012a), which is the most biologically active form of vitamin E, utilized in the human body preferentially to the other forms (Brigelius-Flohé *et al*. 2002). The content in each of the vitamin E isoforms is highly dependent on the cultivar, maturity stage, and geographical origin, but typically detected vitamers include α-tocopherol (8.0–38 mg/100 g dw), α-tocotrienol (0.01–0.30 mg/100 g dw), β-tocopherol (0.02–0.25 mg/100 g dw), γ-tocopherol (0.08–2.1 mg/100 g dw), γ-tocotrienol (0.11–0.24 mg/100 g dw), and δ-tocotrienol (0.02–0.005 mg/100 g dw). Among the various tree nuts, almonds typically contain the most vitamin E (Barreira *et al*. 2012a; Kodad *et al*. 2011; Kornsteiner *et al*. 2006; Madawala *et al*. 2012; Maguire *et al*. 2004; Matthäus & Özcan 2009; Yada *et al*. 2011; Zhu *et al*. 2015), with two handfuls of almonds providing the average daily recommended dose (15 mg) (Institute of Medicine 2000).

Studies analyzing the water-soluble vitamin content of almonds are much scarcer, but these nuts are generally recognized as a good source of riboflavin (vitamin B_2) and other complex B vitamins such as thiamine, niacin, pyridoxine, pantothenic acid, folic acid (folate), and biotin (Yada *et al*. 2011).

Furthermore, almonds are one of the top 40 richest food sources of polyphenols (Pérez-Jiménez *et al*. 2010), which are mainly present as proanthocyanidins, followed by hydrolyzable tannins, flavonoids, and phenolic acids (Bolling *et al*. 2011; Xie *et al*. 2012). Stilbenes are generally available in lower amounts, but these compounds obtained from the shikimate and phenylalanine/polymalonate pathways may also contribute to the health-promoting potential of polyphenol-rich foods, through antioxidant or phytoestrogen activities. In almond, polydatin (Figure 12.1) has been reported as the most abundant stilbene (Xie & Bolling 2014). Furthermore, polyphenols from almond proved to be bioavailable in humans as they were detected as phase II and microbial-derived metabolites in plasma and urine samples (Bartolomé *et al*. 2010).

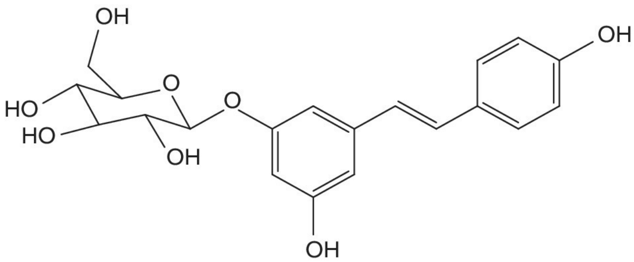

Figure 12.1 Chemical structure of polydatin.

The mineral content of almond, as in other plants, may be affected by many environmental factors and agronomic practices including geographic location, soil composition, water source, irrigation, as well as components of fertilizers and other agronomic production enhancers. Mineral content can also be influenced by the plant species or the botanical component undergoing analysis. In the particular case of almond, the nearly 3% of ash includes mainly potassium (K) > phosphorus (P) > calcium (Ca) magnesium (Mg) >>> iron (Fe) > zinc (Zn) > manganese (Mn), selenium (Se) > sodium (Na) > copper (Cu) (Yada *et al*. 2011).

12.2 *Castanea sativa* Miller (Chestnut)

12.2.1 Botanical Aspects and Geographical Distribution

Castanea sativa Miller belongs to the Fagaceae family, which includes several ecologically and economically important species (Manos *et al*. 2001). Chestnuts are found in three major geographical areas: Asia (with predominance of *C. crenata*, *C. molissima*, *C. seguinii*, C. *davidii*, and *C. henryi*), North America (where *C. dentata*, *C. pumila*, *C. floridana*, *C. ashei*, *C. alnifolia*, and *C. paucispina* thrive) and Europe, where *C. sativa* is predominant (Bounous 2005). According to the Food and Agriculture Organization (FAO), worldwide chestnut production is estimated at about 1.1 million tons. Europe is responsible for about

12% of global production, with relevance for Italy and Portugal, corresponding to 4% and 3%, respectively (Barreira *et al*. 2010). Chestnut is an important food resource in several countries. In Europe, chestnut is regaining interest, with an increase in production area from 81 511 ha in 2005 to 87 521 ha in 2008 (Fernandes *et al*. 2011).

12.2.2 Main Applications and Nutritional Overview

Chestnut kernels are a highly appreciated seasonal nut in Mediterranean countries, being consumed fresh or cooked, with roasting, boiling or frying being the most common cooking methods. Although a highly perishable product, nowadays chestnuts can be found on the market all year round due to the availability of frozen and boiled frozen chestnuts. Other important chestnut products are available on the market, such as the highly appreciated "*marrons glacés*" and chestnut flour obtained by grinding dried chestnuts, used for valorization of small chestnuts or chestnuts with double embryos (Cruz *et al*. 2013).

Chestnuts have become increasingly important in human nutrition because of their nutrient composition and potential beneficial health effects, for example, as part of a gluten-free diet in cases of celiac disease (Pazianas *et al*. 2005) and in reducing coronary heart disease and cancer rates (Sabaté *et al*. 2000). Chestnuts are rich in carbohydrates and are a good source of essential fatty acids (despite their low fat amounts) and minerals, also providing several vitamins and appreciable levels of fiber (Borges *et al*. 2008).

12.2.3 Major Components

On a fresh weight basis, the major component in chestnut is water, which generally accounts for more than 50% of its weight (Barreirra *et al*. 2012b). Starch is the predominant component of dry matter, which makes chestnuts an excellent source of starch, above potatoes or wheat (Borges *et al*. 2008).

Sugar profiles are typically characterized by three main sugars: fructose, glucose, and sucrose. The concentration of each sugar is highly dependent on the cultivar; sucrose was detected as 3.71–24.17 g/100 g dw, glucose varied between 0.96 and 6.81 g/100 g dw, while fructose was quantified between 0.57 and 5.32 g/100 g dw (Barreira *et al*. 2010, 2012b; Bernárdez *et al*. 2004).

Protein content is usually around 3% (2.2–3.1%), depending on cultivar and harvesting year (Barreira *et al*. 2009b, 2012b), mainly constituted by a total of 17 amino acids: cysteine (Cys), proline (Pro), L-alanine (Ala), L-aspartic acid (Asp) (the dominant molecule), glycine (Gly), L-glutamic acid (Glu), arginine (Arg) and the essential amino acids: isoleucine (Ile), leucine (Leu), lysine (Lys), L-histidine (His), L-methionine (Met), L-threonine (Thr), L-phenylalanine (Phe), L-tyrosine (Tyr), L-serine (Ser), and L-valine (Val). Asp is the major amino acid (≈ 1.0 g/100 g dw), closely followed by Glu (≈ 0.8 g/100 g dw), Leu and Ala (≈ 0.6 g/100 g dw) and Arg (≈ 0.5 g/100 g dw). In general, chestnuts are a good source of these compounds; however, amino acid profiles are not well balanced, with certain essential amino acids occurring in limited concentration when compared to FAO recommended levels (Borges *et al*. 2008).

12.2.4 Minor Components

In chestnuts, the fat content is very low, but the fatty acids profile reveals high predominance of unsaturated molecules: 10–20% saturated fatty acids (mainly palmitic acid, C16:0), 10–30% monounsaturated fatty acids (particularly oleic acid, C18:1), and 50–70% polyunsaturated fatty acids (especially linoleic acid, C18:2, and linolenic acid, C18:3) (Barreirra *et al*. 2012b; Borges *et al*. 2007; Fernandes *et al*. 2011). The composition of fatty acids is, as expected, reflected in the triacylglycerol profile: LLLn, LLL, OLLn, PLLn, OLL, PLL, OOL, POL, PLP, OOO, POO, and PPO (L, linoleoyl; Ln, linolenyl; O, oleoyl; P, palmitoyl) (Barreira *et al*. 2012b, 2013). Furthermore, chestnuts are cholesterol free and contain a high amount of vitamin C. Some phenolic compounds, particularly gallic acid and ellagic acid (predominant among hydrolyzable and condensed tannins), and organic acids (oxalic,

cis-aconitic, citric, ascorbic, malic, quinic, succinic, shikimic, and fumaric acids) are also noteworthy (Carocho *et al*. 2013; Gonçalves *et al*. 2010; Ribeiro *et al*. 2007; Vasconcelos *et al*. 2007). Regarding the possible vitamin E isoforms, chestnuts are particularly good sources of γ-tocopherol (γ-tocopherol 754–957 μg/100 g dw; γ-tocotrienol 28–84 μg/100 g dw; δ-tocopherol 39–66 μg/100 g dw; α-tocopherol 4–20 μg/100 g dw; α-tocotrienol 2–8 μg/100 g dw) (Barreira *et al*. 2009a, 2012b; Fernandes *et al*. 2011).

The mineral profile in chestnut is characterized by high contents of K (473–974 mg/100 g dw), P (104–148 mg/100 g dw), Mg (63–93 mg/100 g dw) and Ca (41–51 mg/100 g dw), and low amounts of Fe (5.3–10.9 mg/100 g dw), Mn (3.1–8.0 mg/100 g dw), Na (0.9–3.9 mg/100 g dw), Zn (1.4–3.1 mg/100 g dw) and Cu (1.3–2.7 mg/100 g dw) (Borges *et al*. 2008; Pereira-Lorenzo *et al*. 2005). Concerning human nutritional aspects, chestnuts have an important mineral content. K, Mg, Fe, Mn, and Cu have many physiological functions: K is associated with fluid balance and volume, carbohydrate metabolism, protein synthesis, and nerve impulses; P has an important role in mineralization of bones and teeth, energy metabolism, absorption and transport of nutrients; Mg is important in nervous activity and muscle contraction (Diehl 2002).

Additional information regarding the chemical parameters analyzed in chestnuts can be seen in Table 12.1.

12.3 *Corylus avellana* L. (Hazelnut)

12.3.1 Botanical Aspects and Geographical Distribution

Hazelnut (*Corylus avellana* L.) belongs to the Betulaceae family and is a popular tree nut worldwide, mainly distributed on the coasts of the Black Sea region of Turkey, southern Europe, and in

some areas of the US (Oregon and Washington). Hazelnut is also cultivated in other countries such as New Zealand, China, Azerbaijan, Chile, Iran, and Georgia, among others. Turkey is the world's largest producer of hazelnuts, contributing ≈ 74% to total global production, followed by Italy (≈ 16%), the US (≈ 4%), and Spain (≈ 3%) (Seyhan *et al*. 2007).

Hazelnuts are among the most popular nuts worldwide, with a global production average of nearly 1 million tons (MT) per year (888 328 MT in 2010), on an unshelled basis (Ciemniewska-Żytkiewicz *et al*. 2015).

12.3.2 Main Applications and Nutritional Overview

About 90% of the world crop is shelled and sold as kernels with the remaining 10% utilized in shell for fresh consumption. Besides providing desirable flavor and texture to various foods, hazelnuts can play an important role in human nutrition and health due to their high oil, protein, vitamin, and mineral content (Hosseinpour *et al*. 2013; Tapia *et al*. 2013).

Hazelnuts play a major role in human health due to their very special nutritional value. One hundred grams of hazelnuts provide 600–650 kcal, mainly due to the fat (43–73%), protein (10–25%), and carbohydrate (10–20%) content. Besides being consumed fresh, hazelnuts are also used as an ingredient in confectionery products and the chocolate industry, as raw materials for pastry, and also add flavor and texture to an increasing variety of sweet and savory food products such as bakery, cereal, and dessert formulations (Amaral *et al*. 2006a).

The kernels are commercialized mainly after roasting, which gives them a more intense flavor and a crisper texture. Roasted hazelnuts are usually employed for obtaining butter paste or snacks, and also as ingredients for many products (e.g. cookies, ice cream, breakfast cereals, cakes, chocolates, coffee, bread, liqueurs, and spreads) (Jakopic *et al*. 2011). Eighty percent of the hazelnut kernels are processed in chocolate manufacture, 15% in confectionery, biscuit and pastry manufacture, and 5% is consumed without any further processing (Jakopic *et al*. 2011).

12.3.3 Major Components

Hazelnuts are particularly valuable for their lipid content (Hosseinpour *et al*. 2013; Köksal *et al*. 2006; Parcerisa *et al*. 1998; Venkatachalam & Sathe 2006), with a recognized prevalence of monounsaturated fatty acids, primarily oleic acid, which may reach 80% of total fatty acids. After oleic acid, linoleic acid (10–20%) and plamitic acid (4–10%) are the most abundant fatty acids in hazelnut kernels (Amaral *et al*. 2006a; Köksal *et al*. 2006; Madawala *et al*. 2012; Oliveira *et al*. 2008; Parcerisa *et al*. 1998; Seyhan *et al*. 2007). The lipid fraction is composed of nonpolar (98.8%) and polar (1.2%) constituents. Triacylglycerols are the major nonpolar lipid class, representing nearly 100% of the total nonpolar lipids in hazelnut oil. The main form in hazelnuts is OOO (71–78%), followed by PLL (10–13%), and POO (7.4–11%); other triacylglycerols detected in minor quantities were LLL, OLL, PLL, POL, PPL, PPO, SOO, and PSO (L, linoleoyl; O, oleoyl; P, palmitoyl; S, steareoyl) (Alasalvar *et al*. 2003, 2009; Amaral *et al*. 2006b).

Regarding their protein content, the corresponding amino acids profiles are also noteworthy, with predominance of Glu (2196–3475 mg/100 g) and relevant quantities (400–1000 mg/100 g) of Ala, Asp, Gly, Pro, Ser, and Tyr (Köksal *et al*. 2006).

12.3.4 Minor Components

Hazelnuts are also recognized for their high content of tocopherols, particularly α-tocopherol (19–24 mg/100 g dw), and lower levels of the β (0.6–0.9 mg/100 g dw) and γ (1.3–2.3 mg/100 g dw) isoforms; and sterols, with predominance of *β*-sitosterol (107–126 mg/100 g dw), high content of campesterol (6.7–8.9 mg/100 g dw), stigmasterol (0.7–0.9 mg/100 g dw) and Δ^5-avenasterol (5.1–6.1 mg/100 g dw) and lower levels of cholesterol (0.3–0.9 mg/100 g dw), chlerosterol (0.7–1.2 mg/100 g dw), β-sitostanol (4.4–6.2 mg/100 g dw), Δ^7-stigmastanol (0.21–0.35 mg/100 g dw), campestanol (1.4 mg/100 g dw), fucosterol (0.4–0.6

mg/100 g dw), and Δ^7-avenasterol (0.9–1.3 mg/100 g dw) (Alasalvar *et al*. 2003, 2009; Amaral *et al*. 2006a; Ciemniewska-Żytkiewicz *et al*. 2015; Köksal *et al*. 2006; Kornsteiner *et al*. 2006; Madawala *et al*. 2012; Parcerisa *et al*. 1998). Hazelnuts also contain dietary fiber as well as other beneficial nutrients, such as plant proteins, essential minerals, B complex vitamins, and phenolic compounds (Bignami *et al*. 2005; Köksal *et al*. 2006; Kornsteiner *et al*. 2006).

Besides their rich mineral content, in which K is prevalent (382–1470 mg/100 g dw), followed by P (202–708 mg/100 g dw), Ca (65–401 mg/100 g dw), Mg (35–310 mg/100 g dw), Mn (2.2–19.0 mg/100 g dw), Fe (3.0–5.0 mg/100 g dw), Zn (1.3–4.4 mg/100 g dw), Na (1.2–3.8 mg/100 g dw), Cu (0.9–3.2 mg/100 g dw), chromium (Cr) (10–18 μg/100 g dw), Se (5.5–8.1 mg/100 g dw), and molybdenum (Mo) (2.1–3.8 mg/100 g dw), hazelnut kernels are a valuable source of essential vitamins, such as vitamins B_1, B_6 and niacin (Alasalvar *et al*. 2009; Köksal *et al*. 2006; Seyhan *et al*. 2007).

Several studies characterizing phenolic profiles have been performed (see Table 12.1), revealing significant content of phenolic acids and flavonoids. Several compounds, such as gallic, caffeic, *p*-coumaric, ferulic, sinapic, caffeoyltartaric and caffeoylquinic acids, procyanidins, catechin, epicatechin, glansreginins (Figure 12.2), and phloretins (Figure 12.3), have been quantified in hazelnut samples by several authors (Ciemniewska-Żytkiewicz *et al*. 2015; Jakopic *et al*. 2011; Shahidi *et al*. 2007; Slatnar *et al*. 2014). Phenolics in hazelnut kernels protect the seed against oxidation and are associated with the moderate astringency and characteristic bitter taste of fresh nuts (Slatnar *et al*. 2014).

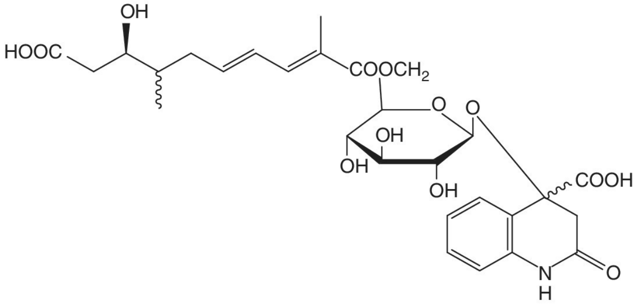

Figure 12.2 Chemical structure of glansreginin A.

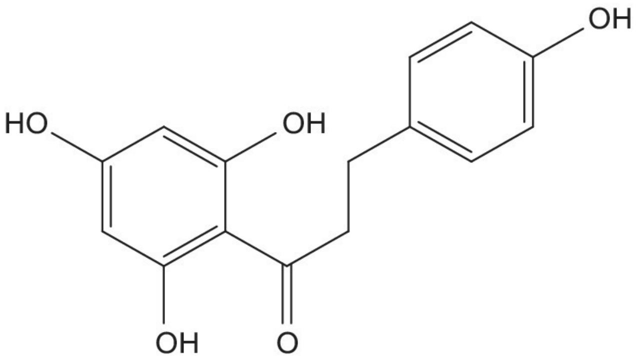

Figure 12.3 Basic structure of phloretin.

Hazelnuts also contain organic acids, but in small quantities, with malic acid as the most abundant compound (Botta *et al*. 1994).

12.4 *Juglans regia* L. (Walnut)

12.4.1 Botanical Aspects and Geographical Distribution

Walnut (*Juglans regia* L.), which belongs to the Juglandaceae family, is a common nut in Mediterranean diets. Originating from Central Asia, the walnut is among the oldest cultivated fruit species. It is commercially planted throughout southern Europe, northern Africa, eastern Asia, the USA, and western South

America. The 2012 world production of in-shell walnut was above 3 400 000 tons (FAO 2013). Recently, walnut has been considered as a natural functional food of high economic interest due to its nutritional and medicinal benefits (Bouabdallah *et al*. 2014; Martínez *et al*. 2010).

12.4.2 Main Applications and Nutritional Overview

Walnut is a crop of high economic interest to the food industry. The edible part of the nut (the seed or kernel) is consumed fresh or roasted, alone or in other processed products. It is a nutrient-rich food mainly due to its high fat and protein content but also contains many vitamins and minerals. The kernel represents between 40% and 60% of the in-shell nut weight. It contains high levels of oil, 52–72%, up to 24% of proteins (usually 13–17%), 1.5–2% of fiber, and 1.7–2% of ash, depending on the cultivar, geographical location, and irrigation rate (Amaral *et al*. 2003; Martinez *et al*. 2006; Prasad 2003).

12.4.3 Major Components

Walnut proteins are highly digestible and have a good balance of essential amino acids. The major protein fraction is glutelins (≈ 70%), followed by globulins (≈ 18%), albumins (≈ 7%), and prolamins (≈ 5%) (Labuckas *et al*. 2014; Sze-Tao & Sathe 2000). The most frequent amino acids in walnut proteins are Arg, Glu, and Ala, but several others are also found, such as Asp, Asn, Ser, glutamine (Gln), Gly, His, Thr, L-citrulline (Cit), γ-aminobutyric acid (GABA), Tyr, Val, Met, tryptophan (Trp), Phe, ornithine (Orn), Ile, Lys, Leu, and Pro (Mapelli *et al*. 2001).

Walnuts contain other beneficial compounds, such as polyunsaturated fatty acids (particularly linoleic acid: 57–66%, oleic acid: 13–24%, linolenic acid: 8–16%, and palmitic acid: 6–11%) and minerals (Bouabdallah *et al*. 2014; Li *et al*. 2007; Rabrenovic *et al*. 2008; Verardo *et al*. 2009). The triacylglycerol profile is characterized by four major molecules: LLLn, LLL, OLL and PLL, but several others were also detected (LLnLn, OLLn,

SLL, OOL, SOL, OOO, SLS, SOO, PLLn, POL, PLS, POO, POS, PLP, and POP) (L, linoleoyl; Ln, linolenyl: O, oleoyl; P, palmitoyl; S, steareoyl) (Bouabdallah *et al.* 2014).

12.4.4 Minor Components

Tocopherols in walnut kernels are dominated by γ-tocopherol (12–39 mg/100 g dw), followed by δ- (1.1–4.6 mg/100 g), α- (0.2–6.6 mg/100 g), and β-isoforms (0.03–0.32 mg/100 g) (Abdallah *et al.* 2015; Kornsteiner *et al.* 2006; Li *et al.* 2007; Madawala *et al.* 2012; Miraliakbari & Shahidi 2008; Verardo *et al.* 2009). The predominant sterol is, by a high margin, β-sitosterol (97–176 mg/100 g), followed by campesterol (0.5–8.8 mg/100 g), Δ^5-avenasterol (0.5–8.0 mg/100 g), and $\Delta^{5,24}$-stigmastadienol (0.8–4.6 mg/100 g). Other detected sterols were cholesterol, brassicasterol, β-sitostanol, Δ^7-campesterol, stigmastanol, $\Delta^{5,23}$-stigmastadienol, Δ^7-stigmastanol, campestanol, stigmasterol, chlenosterol, and Δ^7-avenasterol (Abdallah *et al.* 2015; Amaral *et al.* 2003; Madawala *et al.* 2012; Schwartz *et al.* 2008; Verardo *et al.* 2009).

β-Carotene is the major carotenoid (0.022–0.062 mg/100 g dw), despite the presence of other compounds such as β-cryptoxanthin, lutein, zeaxanthin, violaxanthin, and neoxanthin (Abdallah *et al.* 2015).

The main phenolic compounds in walnut are phenolic acids (chlorogenic, caffeic, ferulic, *p*-coumaric, sinapic, ellagic, and syringic acid), syringaldehyde and juglone (Colaric *et al.* 2005; Zhang *et al.* 2009), besides several hydrolyzable tannins and different flavonoids (especially vescalagin) (Fukuda *et al.* 2003; Slatnar *et al.* 2015; Verardo *et al.* 2009). Recently, more than 120 phenolic compounds, including hydrolyzable and condensed tannins, flavonoids and phenolic acids, have been identified or tentatively characterized in different walnut cultivars (Grace *et al.* 2014; Regueiro *et al.* 2014).

Walnuts are also characterized by high levels of K (300–487 mg/100 g), Mg (129–443 mg/100 g dw), P (308–385 mg/100 g dw),

and Ca (58–135 mg/100 g dw) and, in contrast, very low levels of Na (0.3–6.7 mg/100 g dw), Mn (1.1–4.3 mg/100 g dw), Fe (1.5–2.9 mg/100 g dw), Cu (0.7–2.0 mg/100 g dw), Zn (1.2–1.9 mg/100 g dw), and Se (0.7–1.1 µg/100 g dw) (Lavedrine *et al*. 2000; Tapia *et al*. 2013).

In all these examples, laboratory determinations were achieved by applying several methodologies (see Table 12.1).

12.5 Conclusion

Nut consumption as part of a balanced diet is recommended. Clinical and preclinical trials have demonstrated that nuts have antioxidant, antidiabetic, and hypocholesterolemic actions (Xie & Bolling 2014). Furthermore, their consumption may improve body weight control and reduce the risk of obesity-related diseases such as coronary heart disease and type 2 diabetes. In addition to cardiovascular benefits, which are mainly due to the lipids present in many types of nuts, other components might have important protective roles against the onset of several diseases.

Given the described profiles of different phytochemicals, it is also advised to consume a high variability of nuts, since their potential effects are often complementary, in relation to their different compositions of major and minor components.

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13
The Contribution of Chestnuts to the Design and Development of Functional Foods

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13.1 Introduction

Chestnut (*Castanea sativa* Miller), a fruit traditionally consumed and appreciated by Europeans, has been studied and exploited industrially as an ingredient in functional foods. It has prebiotic, antioxidant, and cardioprotective properties, and is an excellent source of bioactive compounds such as starch and fibers, which can contribute to meeting the nutritional needs of different consumer groups. Chestnut belongs to the Fagaceas family, particularly to the genus *Castanea*. It originated in Asia Minor, where it is known as *kashtah*. The Greeks probably introduced it into Europe and its name came from the Latin word *castanea*, which derives from the ancient Greek word *kastanon*. The genus *Castanea* comprises 13 species of chestnut trees, but only four have commercial interest: *Castanea crenata* Siebold & Zucc (Japan), *Castanea dentata* (Marshall) Borkh (North America), *Castanea mollissima* Blume (indigenous to China), and the already mentioned *Castanea sativa* (Europe) (Lage 2006). In addition to the frequent consumption of this fruit in its fresh form, the bioactive properties of some of its compounds justify the great interest of the food industry. Therefore, this chapter will address aspects of its composition and functional properties. These topics are essential for the definition of technologies in the design and development of functional foods, which in addition to promoting consumer health can improve production chain operations in a sustainable way.

13.2 Chestnut Composition

The chestnut tree bears fruits and contributes to scenic beauty in mild climates. Chestnut (Figure 13.1) is a fruit composed of pericarp (outer shell), integument (inner shell), and a distinct endocarp layer surrounding the edible seed.

Figure 13.1 The commercial chestnut.

In general, it can be said that chestnuts have more carbohydrates than fat. In addition to the popular consumption of cooked (boiled) chestnuts, they are widely used as an ingredient in baking and the confectionery industry. Table 13.1 shows the chestnut proximate composition compared with other widely consumed nuts and peanut. It can be seen that, for example, its carbohydrate content is higher than that of the Brazil nut.

Table 13.1 Proximate composition of chestnuts compared to nuts and peanut.

Nutrients (g/100 g)	Chestnut[a]	Brazil nut[b]	Cashew nut[b]	Macadamia[b]	Peanut[b]
Moisture	52.9	3.10	4.39	2.10	6.20
Total fat	2.63	64.94	42.06	66.16	44.57
Crude protein	6.51	14.11	14.11	8.40	24.03
Nitrogen	NI[c]	2.62	3.55	1.58	3.95

Total carbohydrate	38.6[d]	6.27	6.27	22.18	12.01
Total dietary fiber	13.7[e]	8.02	1.42	NI	11.30
Ash	2.06	3.56	2.66	1.16	1.89

[a] Chestnut (*Castanea sativa* cv. Judia) (Borges *et al*. 2008).

[b] Freitas and Naves (2010).

[c] Not reported.

[d] Referred to as starch.

[e] Reported as "total fiber."

An important aspect highlighted in the literature is the differences in nutritional composition between *C. sativa* cultivars (cvs). Table 13.2 shows the nutritional composition of chestnut according to different authors.

Table 13.2 Nutrients of some *C. sativa* cultivars (de Vasconcelos *et al*. 2007).

	Composition (g/100 g dry weight)				
Cultivar	**Dry matter**	**Starch**	**Crude protein**	**Crude fat**	**Total ash**
Judia	50.37 ± 1.54	64.86 ± 1.63	4.87 ± 0.33	1.72 ± 0.39	2.34 ± 0.20
Longal	53.87 ± 3.83	64.15 ± 3.50	5.13 ± 0.43	1.56 ± 0.31	1.91 ± 0.05
Martainha	48.73 ± 3.01	64.82 ± 1.33	3.89 ± 0.13	1.89 ± 0.51	1.87 ± 0.20

It is important to emphasize that the large variability among the different cvs can sometimes make it difficult to describe their chemical characteristics. This is due to several reasons:

- some data refer to chestnuts and others to *marrons*, which are products with different morphological traits and technological

characteristics

- some varieties (e.g. *Marrone fiorentino*) have different ecotypes with different chemical characteristics linked to their ecological surroundings
- different clones of the same variety can show different chemical composition
- chestnut composition is considerably associated with the harvesting year, and the association between year and cultivar is also significant (Neri *et al*. 2010).

Regarding the influence of environmental conditions, chestnuts from Spain show high variability between cvs and regions, in terms of proximate composition. Correlations with environmental parameters were low, indicating that the differences found between regions probably reflected the differences between the cvs. In central and southern Spain, some *C. sativa* cvs showed lower moisture content, probably due to the low summer rainfall in these regions (Pereira-Lorenzo *et al*. 2006).

Barreira *et al*. (2010b) demonstrated the high variability between cvs. The sugar profile of different chestnut cvs from Portugal (*C. sativa*) revealed high heterogeneity. Sucrose was the main free sugar (g/100 g dry weight (dw)) in the cvs (Aveleira 22.05 ± 1.48, Judia 23.30 ± 0.83, and Longal 9.56 ± 0.91), while glucose was slightly more prevalent in Boa Ventura cvs (6.63 ± 0.49). Chestnut starch granules are oval to round with an average size ranging between 9 and 13 mm. To the naked eye, an isolated chestnut granule appears to be a white powder (Correia *et al*. 2012).

Regarding mineral composition, de Vasconcelos *et al*. (2010a) reported that potassium and phosphorus were predominant in different cvs from different harvest years. Calcium, magnesium, iron, zinc, and manganese were also present.

Significant levels of lutein, β-carotene, γ-tocopherol, and vitamin C were also found in chestnut fruits. As for the lipids, Borges *et al*. (2007) reported that chestnut cvs from Portugal have a low crude fat content, low saturated fatty acids (SFA) (17%), and high unsaturated fatty acids (UFA) (83%). Linoleic, oleic, and palmitic acids were the major fatty acids found, which accounted for more

than 85% of the total FA content of chestnuts. Also, chestnuts contain health-promoting compounds, including antioxidants such as vitamin C and E, carotenoids, and polyphenols (mainly gallic and ellagic acids). Chestnut cvs from different provinces in the Anatolia region showed that total phenolic content varied between 5 mg gallic acid equivalent (GAE)/g dw and 32.82 mg GAE/g dw (Otles & Selekt 2012).

Other researchers have evaluated chestnut processing stages, such as drying, and found that it seemed to negatively affect fruit composition, especially by reducing its amino acid content. At all processing stages, the fruits contained low values of protein (4.1–5.4 g/100 g dw) and the essential amino acid threonine had the highest value (3.6–10.0 mg/100 g) (de Vasconcelos *et al.* 2009). Whatever the nutrient content of chestnuts, cooking processes significantly affect their metabolites. Gonçalves *et al.* (2010) studied the effects of processing (roasting and boiling) on the primary and secondary metabolite composition of different chestnut cultivars from three Protected Designation of Origin (PDO) areas in Trás-os-Montes and Alto Douro provinces in Portugal. They reported that cooking processes significantly affected the primary and secondary metabolite composition. Roasted chestnuts had higher protein content, insoluble and total dietary fiber, and lower fat content, whilst boiled chestnuts had lower protein and higher fat contents. Cooking increased citric acid content, especially in roasted chestnuts. The observed increases in organic acids could be explained as heat-induced reactions between nitrogen-free carboxylic acids and sugars. On the other hand, raw chestnuts had higher malic acid content than cooked (boiled) chestnuts. Roasted chestnuts had significantly higher gallic acid and total phenolic contents, and boiled chestnuts had higher gallic and ellagic acid contents when compared to raw chestnuts. These data confirmed that cooked chestnuts are a good source of organic acids and phenolic compounds and have low fat content, properties associated with health benefits.

Concerning other food products, the chestnut is used as an ingredient of a commercial brand of vegetable milk. It presented 2.4% of dietary fiber, higher than hazelnut and walnut milks, which contain 0.4% and 0.9%, respectively (Bernata *et al.* 2014). Therefore, it is important to mention that in spite of the several

factors that affect its chemical composition, such as differences between the cvs, the high nutritional value of chestnut supports its potential use in functional foods.

The high consumption levels of the fresh chestnut are probably related to its nutritional composition, organoleptic value and the increasing consumer interest in organic products. In Portugal, for example, not only is the fruit regularly consumed, but chestnut flour is also widely used. Some ancient documents report that in the Middle Ages chestnut was used as the main ingredient in bread production and as a kind of porridge (Lage 2003).

The popularity of dietary ingredients reported to improve human health has increased in recent years. The chestnut is well known as a carbohydrate and fiber source. According to the EFSA (2010), the glycemic carbohydrates provide carbohydrate to body cells, mainly in the form of glucose. The main glycemic carbohydrates are glucose and fructose (monosaccharides); sucrose and lactose (disaccharides); maltooligosaccharides; and starch (polysaccharide). Dietary fiber is defined as nondigestible carbohydrates plus lignin. The EFSA considers that the main types of total dietary fiber are:

- nonstarch polysaccharides (NSP) – cellulose, hemicelluloses, pectins, hydrocolloids (i.e. gums, mucilages, glucans)
- resistant oligosaccharides – fructooligosaccharides (FOS), galactooligosaccharides (GOS), other resistant oligosaccharides
- resistant starch – consisting of physically enclosed starch, some types of raw starch granules, retrograded amylose, chemically and/or physically modified starches
- lignin associated with the dietary fiber polysaccharides.

The terms “soluble” and “insoluble” dietary fiber have been used in the literature to differentiate between viscous, soluble types of fibre (e.g. pectins) and insoluble components such as cellulose.

According to de Vasconcelos *et al.* (2010b), the mean of total carbohydrates was estimated to be 44.7 g/100 g fresh chestnut fruits. Therefore, the average crude protein content in fresh chestnut has been estimated to be 3.5 g/100 g. Moreover, chestnuts can be used as an important source of dietary energy. The average

energy content in fresh chestnut fruits has been estimated to be 198 kcal/100 g. Considering the major chestnut nutrients, it is worth mentioning that each consumer group should focus on satisfying its daily needs taking into consideration the intake of this fruit associated with other dietary foods.

13.3 Biotechnology and Safety

Issues related to health safety and use of biotechnology in chestnut production have been addressed in order to protect the tree and fruits and provide data on the sustainability of this species. The genetic variability of Turkish chestnut was studied in 13 chestnut populations. The results were compared with existing data on Italian chestnut populations, and western Turkish demes seem to be more closely related genetically to Italian populations than to eastern demes (Villani *et al*. 1991). The genetic linkage map of European chestnut was studied in 96 individuals, and the findings were used as a starting point for studies on the structure, evolution, and function of the chestnut genome (Casasoli *et al*. 2001). Chestnut quality is associated not only with the nutritional characteristics of the variety, taste, and size, but with the absence of parasites and microbiological contamination. The most common insect pests of chestnut in European cultivars are some species of *Lepidoptera tortricidae* and *Coleoptera curculionidae*, against which different biological control techniques have been tested.

Since they are perishable and susceptible to water loss, chestnuts are also susceptible to fungal attack (Vinciguerra & Clausi 2006). Some Fungi species such as *Penicillium* spp. preferred specific chestnut cvs (Sieber *et al*. 2007). Kačaniova *et al*. (2010) studied the plant–microbial interactive relations with respect to determination of the mycoflora of chestnut tree, nuts, shell, leaves, and pollen and their effect on the host organism in four Slovak regions. In this study, seven genera and 10 species of microscopic fungi were isolated from the nutshell and leaves. *Alternaria*, *Cladosporium*, *Mucor*, and *Rhizopus* appeared to be the most frequently occurring genera on nuts, leaves, and shell. It was found that isolates from chestnut pollen were represented by eight genera

and 11 species of microscopic fungi, *Acremonium, Alternaria, Cladosporium, Fusarium, Penicillium*, and *Trichoderma* being the most common fungi found. It is necessary to emphasize that they are considered the most important producers of mycotoxins, a hepatotoxic metabolite of some fungi. Chestnut samples timed from harvest to the end of the storage period were mainly contaminated with the genera *Fusarium*, *Cladosporium*, *Alternaria*, and *Penicillium*, although very small numbers of the genus *Aspergillus* were isolated (Rodrigues *et al*. 2013). These fungi were determined to be moderately associated with mycotoxin production, which can be indicative of mycotoxin co-contamination problems, if adequate storage conditions are not secured. Fungi and mycotoxins can be harmful to human health, and thus their presence in the chestnut production chain should be prevented to ensure the supply of high-quality and safe raw material to the food industry.

13.3.1 Functional Properties and Health Effects

Product design and development require studies involving not only the chemical composition of chestnuts, but also determination of the most appropriate technology that makes production with higher added value possible. In addition to the high consumption of cooked (boiled) chestnuts due to their nutritional and organoleptic potential, the food industry has shown growing interest in new products derived from chestnuts. These products are developed focusing on functional and health-promoting properties. In general, they can be classified as antioxidants, prebiotic, and gluten-free products.

13.3.1.1 Antioxidants

Epidemiological studies indicate that fruits and vegetables offer protective effects against degenerative conditions such as cancer and cardiovascular diseases. Since oxidative stress is a common pathway of chronic degenerative diseases, it has been assumed that dietary antioxidants may explain this protective effect (Blomhoff *et al*. 2006). Of the tree nuts, walnuts, pecans, and chestnuts have

the highest content of antioxidants. Among the various antioxidants present in chestnuts, the most abundant is L-ascorbic acid, the biologically active form of vitamin C.

Barros *et al*. (2011) evaluated the total vitamin C content and antioxidant activity of raw and cooked chestnuts. The vitamin C content of raw chestnuts varied significantly between the different cvs studied. Different cvs behave differently during the cooking process in terms of vitamin loss. A significant decrease in the vitamin C content was observed during the boiling process (25–54%) and roasting process (2–77%). Moreover, the cooking process significantly changed the antioxidant activity of the chestnuts. Differences in antioxidant activity were also observed between the cvs during the cooking processes. The variation in the vitamin C content of raw chestnuts explains 99% of the antioxidant activity variation, but in roasted and boiled chestnuts this percentage significantly decreases to 51% and 88%, respectively. Although high antioxidant activity is still present in cooked (boiled) chestnuts, it is less dependent on its vitamin C content, probably due to the conversion of ascorbic acid to dehydroascorbic acid. Neri *et al*. (2010) studied the composition of three commercial Italian sweet chestnut (*C. sativa* Mill.) ecotypes, Marrone di Castel del Rio, Marrone di Marradi, and Marrone di Valle Castellana, from Emilia Romagna, Tuscany, and Abruzzi region, respectively. The content of antioxidant compounds (ascorbic acid and total polyphenols) and the antioxidant activity of the nuts were investigated for two consecutive years. These authors indicated that all the ecotypes showed low polyphenol content but were high in ascorbic acid, which accounted for a discrete antioxidant activity (3.02–3.11 trolox-equivalent antioxidant capacity (TEAC)/g fw) in the nuts.

The increase in gallic acid during the cooking process, which was presumably transferred from the peel to the fruit, also contributes to the high antioxidant activity of cooked chestnuts (Braga *et al*. 2015).

Therefore, chestnuts could be a natural substitute source for the antioxidants currently added to foods and animal feed. Moreover, they could be useful in pharmaceutical products such as antibiotics due to their potent antibacterial activity and antioxidant capacity

(Heung Sung *et al.* 2012).

Several studies have been conducted on chestnut by-products, namely leaf, shell and bur, revealing them to be good sources of phenolic compounds with marked biological activity, mainly antioxidant properties (Braga *et al.* 2015). Moreover, topical application of antioxidants such as vitamins C and E has been proven to be effective in the protection of skin against UV-mediated damage. Almeida *et al.* (2008) performed a study evaluating the topical application of ethanol/water extracts from chestnut leaves and concluded that a strong absorption at 280 nm could forecast a possible effectiveness of chestnut leaf extract topical administration to prevent UV radiation-induced skin damage.

Chestnut shell extracts are rich in phenolic compounds, mainly phenolic acids and tannins (condensed and hydrolyzable). Furthermore, extracts from chestnut flower, leaf, skins, and fruit were evaluated using several biochemical assays, including inhibition of oxidative hemolysis in erythrocytes and inhibition of lipid peroxidation. Chestnut skins (inner and outer) revealed a good antioxidant capacity and a high content of polyphenols and flavonoids, demonstrating a direct correlation between antioxidant capacity and the concentration of these bioactive compounds (Barreira *et al.* 2008). In other research, chestnut skins and leaves were evaluated (*C. sativa* Mill. cvs Aveleira, Boa Ventura, Judia, and Longal) and demonstrated better results compared to almond green husks (*Prunus dulcis* L. cvs Duro Italiano, Ferraduel, Ferranhês, Ferrastar, and Orelha de Mula). The chestnut by-products proved to have a high potential for application in new antioxidant formulations (Barreira *et al.* 2010a).

The chestnut shell waste products from the food industry were analyzed as a potential source of antioxidant compounds. Compared with other waste products, such as eucalyptus (*Eucalyptus globulus* Labill) bark, the extraction yield, antioxidant activity, and total phenolic content of the extracts were higher in the chestnut shell than in the eucalyptus bark for most of the extraction conditions assayed (Braga *et al.* 2015). Vazquez *et al.* (2008) reported that extraction of chestnut shell with a 2.5% Na_2SO_3 aqueous solution, comparing with eucalyptus, resulted in

the highest extraction yield: 25.6%, total phenols 13.4 g GAE/100 g oven-dried shell and ferric reducing antioxidant potential (FRAP) antioxidant activity of 80.7 mmol ascorbic acid equivalent/100 g oven-dried shell. A positive linear correlation could be established between antioxidant activity and total phenolic content of these extracts. Moreover, Fourier transform infra-red (FTIR) spectroscopy confirmed the higher content of phenolic compounds in the chestnut shell extracts compared to the eucalyptus bark extracts. Chestnut shell extracts were characterized by the presence of high molecular weight compounds, whereas lower molecular weight compounds were predominant in eucalyptus bark extracts (Vazquez *et al.* 2008).

13.3.1.2 Prebiotics

Prebiotics are defined as nondigestible food ingredients that beneficially affect the host by stimulating the growth and/or activity of one or a limited number of bacteria such as probiotic bacteria in the colon, thus improving host health. These compounds include some soluble fiber compounds such as fructooligosaccharides and α-oligosaccharides, as well as inulin, resistant starch, polyols (lactitol, mannitol, sorbitol, xylitol) and modified dextrins (Siro *et al.* 2008).

Most foods are multicomponent systems that contain complex mixtures of water, carbohydrates, proteins, lipids, and minor constituents. Starch, for example, is a macroconstituent of many foods, and its properties and interactions with other constituents are of interest to the food industry. Globally, intensive efforts have been concentrated on producing polysaccharide derivatives of different types of starch for diverse industrial applications. The widespread use of starch is justified because it is inexpensive and available in large quantities. In addition, it is relatively pure and does not require intense purification, as is often the case with other natural polymers such as cellulose and gums. Commercial starches are obtained from cereals, such as corn, wheat, and various types of rice, and from tubers or roots, such as potato and cassava (or tapioca).

Chestnut is an excellent source of resistant starch, which can be

defined as the starch that cannot be digested in the small intestine of healthy individuals. Like fibers, resistant starch contributes to the lower glycemic index of foods inducing a lower glycemic response and, consequently, lower insulin response. Thus, it can be helpful in the treatment of diabetes (Pereira 2007).

Several studies have been carried out on chestnut as a functional food. Torres *et al*. (2014) studied the particle size distribution, color, morphology, and chemical composition of starches isolated from fresh chestnut fruits (S1), semidried chestnut fruits at room temperature (S2), and commercial chestnut flour (S3). There were significant differences in the total starch content, and starch isolation was more selective in the dried samples. All samples showed low damaged starch (<2.91%) and intermediate amylose (from 17.0% to 25.8%) content on a dry weight basis. The lowest amount of amylose was found in S1, but it was within the range of common commercial starches. Pizzoferrato *et al*. (1999) studied changes in chestnut starch in terms of structure and digestibility in order to understand the changes caused by cooking and, specifically, by the Maillard reaction. The results revealed major changes in the macromolecular structure of starchy materials and that these changes are correlated with digestibility changes in terms of enzymatic degradation resistance. In the system studied, the extent of the Maillard reaction was not great enough to exert a significant influence on structure and/or digestibility of the chestnut starches.

Different chestnut-based food products with prebiotic properties have been developed, such as chestnut puree which was developed in order to use the seasonal surplus of overproduction, providing, at the same time, a response to the growing demand for healthy and environmentally friendly products. These purees, prepared with broken dried chestnuts, are fermented with six different strains of *Lactobacillus rhamnosus* and *Lactobacillus casei*. Conventional *in vitro* tests have indicated the six lactobacilli strains as promising prebiotic candidates; moreover, the fact that these strains were able to grow and survive in chestnut puree at a population level higher than 8 $\log_{10}$ colony-forming unit (CFU)/mL during 40 days of storage at 4 °C demonstrated the prebiotic properties of these purees. This was the basis for the production of a new food, lactose free and with reduced fat content (Blaiotta *et*

al. 2012).

13.3.1.3 Gluten-Free Products

Celiac disease is a permanent intolerance to different gluten formers in cereal proteins such as wheat gliadin components, rye prolamin, barley hordein, and oat avidin. Nearly 1% of the world's population has celiac disease (Kiskini *et al*. 2007). Gluten intake by celiac people leads to inflammation of the small intestine and lack of absorption of important nutrients, such as iron, calcium, folic acid, and fat-soluble vitamins.

Sufferers of celiac disease have to stick to a gluten-free diet, which limits the type of foods they can consume. Chestnut is a gluten-free food so many new products derived from chestnuts and chestnut flour have been developed to replace wheat/cereal-containing foods. Chestnut flour, for example, contains high-quality proteins with essential amino acids, adequate amounts of sugar (13.9–32.6%), starch, and dietary fiber (4–10%), low amount of fat, vitamins E and B group, and essential mineral elements such as potassium, phosphorus, and magnesium (Chenlo *et al*. 2007). This flour can be used in the production of gluten-free breads (GFBs) leading to good nutritional quality and health benefits. Demirkesen *et al*. (2010) developed GFB formulations using a blend of chestnut and rice flours, at different mixing ratios (0/100, 10/90, 20/80, 30/70, 40/60, 50/50, and 100/0). The effects of different hydrocolloids such as locust bean/xanthan gum and guar gum/xanthan blends and emulsifiers on the rheological properties of dough formulations and on the bread quality were reported. Due to the rigid and compact structure of the fibrous chestnut flour, the bread formulated using only chestnut flour had the hardest structure and the lowest volume. The loaf volume decreased with the increase in chestnut flour content. This may be due to the fact that the high sugar content of chestnut flour led to reduced starch gelatinization and resulted in low specific volume and hardness of breads.

13.3.2 Functionality of Chestnut Products

There are many aspects that can affect the functionality of chestnut products, such as temperature and oxygen. Tzortzakis and Metzidakis (2012) studied the effects of heat stress (HS) and ultra-low oxygen (ULO), under controlled (CA: continuous airflow of 40 mL/min) or modified (MA: in commercial sealed bags with 5 L capacity which were aerated biweekly) atmospheres on chestnut (cv. Rodiana) quality and storability. Chestnut fruits were exposed to ULO (1% O_2 for 1 h) or dipped in a water bath (at 55 °C for 15 min), and then stored under CA or MA at 6 °C for up to 90 days. The HS dipping and storage under CA or MA increased sprouting (up to 60%) and mold significantly in chestnut samples comparing with the control treatment (CA). Under MA, HS and ULO increased respiration rate. Total starch content increased (up to 30%) in MA-HS and MA-ULO treatments compared with the control treatment during the first 60 days of storage. Chestnut moisture content decreased during the first 30 days of CA and MA storage. No major differences were observed in total sugar, total fat, and total phenolic content. The chestnut fruits were intact without any obvious wormholes. In the sensory evaluation, 57% of panellists identified differences between the treatments. They showed greater preference (67%) for the chestnut treated with HS and stored under MA. Additionally, MA-HS enhanced chestnut appearance (up to 30%), while no differences were observed between the treatments and different storage conditions for aroma, sweetness, and texture.

Irradiation is another technology applied as an alternative preservation method for food. Antonio *et al.* (2011) evaluated the influence of γ-irradiation on the antioxidant potential of chestnut fruits and skins. Their findings indicated that this storage method favored chestnut antioxidant potential. The application of γ-irradiation also seems to be advantageous for antioxidant activity, independently of the dose used (0.27 ± 0.04 kGy or 0.54 ± 0.04 kGy). Carocho *et al.* (2012) reported the effect of electron beam and γ-irradiation (doses of 0, 0.5, 1, and 3 kGy) on the antioxidant potential of Portuguese chestnuts. Irradiated samples preserved total phenolic content (but not flavonoids) and revealed higher antioxidant activity (lower EC_{50} values) than the control samples. The most indicated doses to maintain antioxidant content and increase antioxidant activity were 1 and 3 kGy for electron beam

and γ-irradiation, respectively.

The drying stage in the processing of chestnuts seems to be the key for property stability. In dehydrated chestnuts, the initial moisture content (mc) of about 50% (100% dry basis) decreased to a final moisture content of 7.4% (8% dry basis). The results showed that drying air temperature significantly influenced the total drying time, and air velocity influenced the total energy requirement for drying (Koyuncu *et al.* 2004). Koyuncu *et al.* (2004) also found that the minimum and maximum energy requirements were 6.47 and 25.25 kWh/kg for drying chestnuts at 50 °C and 0.5 m/sec and at 40 °C 1.0 m/sec, respectively. Correia *et al.* (2009) studied the effect of using different drying conditions on the morphological and chemical properties of two Portuguese chestnut cvs (Longal and Martainha). All chestnut drying curves were found to be different according to the drying temperatures used (40 °C, 50 °C, 60 °C, and 70 °C). These conditions also affected both the chemical composition of flours and the morphological properties of starch. In general, the color parameters of the flours decreased with increased drying temperature, and the total color difference also significantly changed in the samples dried under the different conditions evaluated. The results showed that the higher the drying temperature, the higher the reducing sugars content and the lower the starch content.

De Vasconcelos *et al.* (2007) analyzed the composition of health-related compounds of chestnuts from different cvs at different stages of industrial transformation to detect both primary and secondary metabolites. The samples (Longal, Judia, and Martainha cvs) were collected at the end of each processing step: (a) fresh fruits, (b) after two months of storage at 0 °C, (c) after industrial steam peeling, and (d) after freezing with liquid air and -20 °C storage. All three cvs had a significant content of polyphenols with gallic acid. The authors reported that ellagic acid was predominant among the hydrolyzable and condensed tannins. As for the fresh chestnuts, the results showed significant differences between the three cvs in most of the parameters studied.

Despite its rich composition, processing stages such as drying and freezing seem to affect the product. Different chestnut cvs were evaluated, and the results showed that the sugars were the most

affected by the processing stages. Significant levels of lutein, lutein esters, γ-tocopherol, and vitamin C were reported in the chestnut fruits. On the other hand, fruit carotenoids and vitamin C significantly decreased during industrial processing.

13.4 Conclusion

Due to increased concern about the effects of food on consumer health, many studies have been carried out on chestnut focusing not only on its chemical composition, but also on its benefits to human health. Several of these studies have looked at major nutrients such as fat and carbohydrates, especially starch; therefore, there is a need for further studies on other substances such as polyphenols and the effects of processing stages at industrial scale on the properties of chestnuts.

The content of chestnut starch can have a wide range of applications due to its pasty texture, associated with a high amylose content and strong, elastic, and stable gels. These properties give the native chestnut starch the ability to improve the texture of foods, such as noodles, and provide viscosity and adhesion and binding properties during the food production process. In addition to the nutritional factors, the development of new chestnut products with functional properties can be undertaken effectively in terms of lower costs, reducing the negative impact of waste on the environment and providing other economic benefits to businesses.

Chestnut has aroused interest for the development of new products but this is not only in terms of the fruit but also the processing waste, for example leaves and shell. These residues can be used as sources of bioactive substances such as phenolic compounds, flavonoids, and tannins since some of them have been shown to have beneficial effects in preventing diseases such as diabetes and cardiovascular diseases.

Chestnuts can provide countless benefits to human and animal health. However, improvements can still be made in production processes and quality and in terms of genetic selection with optimization of industrial processing while maintaining a

sustainable production chain.

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14
Emerging Functional Foods Derived from Almonds

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14.1 Introduction

The development of functional foods with constituents that exert beneficial bioactions in health promotion and prevention is a field in expansion. The increase in their developments is attributed mainly to the association of their consumption with reduced risk of chronic diseases (Shahidi 2009). This great attribute of functional foods in health promotion and prevention is very appealing to consumers with an awareness of maintaining/promoting health through foods. Thus, the demand for functional foods is increasing because consumers believe that foods can contribute directly to their health and life quality (Betoret *et al*. 2011; Siegrist *et al*. 2008; Siró *et al*. 2008).

Functional foods are spread among all different food sectors, from breakfast cereals to dairy products and from processed meats to beverages. Functional ingredients in both original and derivative forms can be incorporated into a wide range of food products. Popular functional ingredients include fibers, polyphenol-rich extracts, and food with well-known human health benefits such as berries and dark cocoa. In recent decades, tree nuts have been recognized as a food group with multiple health benefits, ranging from cholesterol reduction to blood glucose control. Among all tree nuts, almonds have been regarded as the epitome of healthy foods because they are a rich source of protein, monounsaturated fatty acids, dietary fiber, vitamin E, riboflavin, and essential minerals as well as phytosterols and polyphenols (Kendall *et al*.

2010; Yada *et al*. 2013). All of these nutrients/nonnutrients and other unidentified constituents work together in a synergistic manner to make almonds an ingredient ready for incorporation into functional foods. There is a great body of clinical evidence showing that almond consumption is inversely associated with several risk factors for chronic disease, i.e. dyslipidemia, hyperglycemia, oxidative stress, inflammation, and overweight/obesity. While the clinical evidence still needs to be gathered, fiber and polyphenols in almonds with bacteria-modulating properties may also help maintain/promote gut health by serving as prebiotics.

This chapter will provide an overview of the diverse bioactions of almonds and their nutrients and discuss how almonds can be used in the development of functional products.

14.2 Overview of Almond Nutrients

Almonds are a nutrient-dense food, as defined by the US Food and Drug Administration (FDA) because of the rich content of multiple nutrients (Chen *et al*. 2006). Almonds are an excellent source of magnesium and α-tocopherol (containing >20% of the daily value (DV) (FDA 2013)) and a good source of protein, phosphorus, fiber, copper, and riboflavin (containing 10–20% DV in one serving – around 28 g) (Figure 14.1).

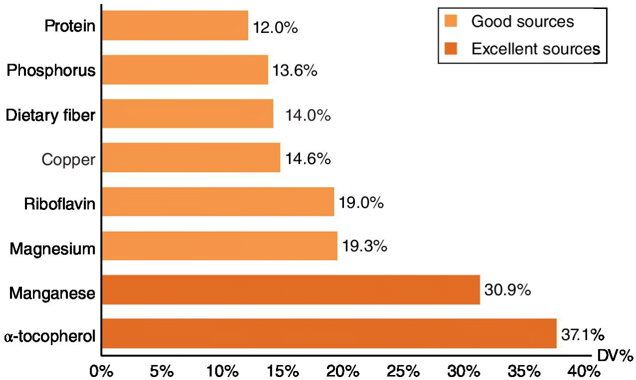

Figure 14.1 The percentage of daily value (DV) of the selected nutrients in 28 grams (1 serving) of almonds.

The energy provided by almonds is derived largely from the fat content, ranging from 25 to 66 g/100 g fresh weight (Yada *et al*. 2011). It is worth noting that fats in almonds comprise mainly monounsaturated fatty acids (MUFA) at 63.2%, and polyunsaturated fatty acid (PUFA) at 24.7% (USDA 2014). Further, almonds are free of cholesterol. Like olive oil, oleic acid is the predominant fatty acids in almonds. Also, almonds are appreciated as a vitamin-rich food because one serving (28 g) can provide half the recommended daily amount (RDA) (Hellwig 2006) of α-tocopherol (7.5 mg). Almonds are also packed with many B vitamins, e.g. riboflavin, niacin, thiamine, pantothenic acid, pyridoxine, and folates. Polyphenols, which display an array of bioactions, including antioxidation, antiinflammation, and glucoregulation, have been characterized in almonds (Milbury *et al*. 2006). Polyphenols are mainly present in the skins but the content varied widely between cultivars, ranging from 127 (Fritz) to 241 (Padre) mg gallic acid equivalent/100 g of fresh weight (Milbury *et al*. 2006). Among flavonoids, flavanols and flavonol glycosides were the most abundant, comprising up to 38–57% and 14–35% of the total quantified polyphenols, respectively (Monagas *et al*. 2007).

14.3 Health Benefits and Bioactions of Almonds

Almonds and other tree nuts and peanuts were previously considered unhealthy foods, mainly due to their high fat content, which may cause unwanted weight gain. Since the early 1990s, the health benefits of their consumption have been increasingly documented in clinical trials. Almonds are associated with a reduction in blood cholesterol and glucose, biomarkers of oxidative stress and inflammation. Further, they can be incorporated into dietary regimes for weight loss or maintenance. The putative mechanisms by which almonds and their constituents protect against risk factors of chronic diseases are demonstrated in Figure 14.2. All of this evidence supports the recommendation for

their incorporation into functional foods.

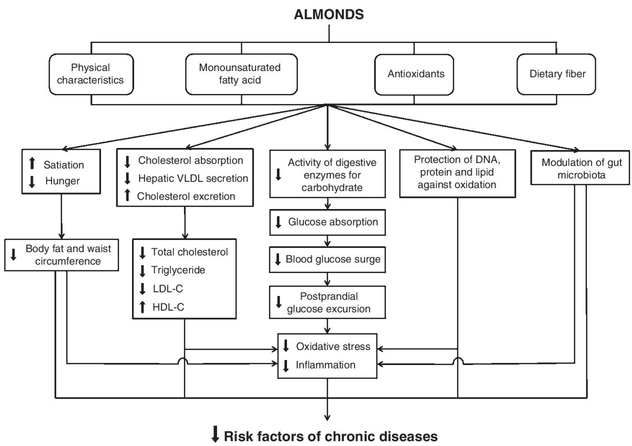

Figure 14.2 Putative mechanisms by which almonds and their constituents protect against risk factors for chronic diseases.

14.3.1 Cholesterol Reduction

In recent decades, many clinical trials have consistently demonstrated that almonds are beneficial to blood cholesterol control, lipid profile, and lipoproteins in different populations, including healthy individuals (Abbey *et al.* 1994; Berryman *et al.* 2015; Hyson *et al.* 2002; Jaceldo-Siegl *et al.* 2011) and patients with hypercholesterolemia and diabetes (Damasceno *et al.* 2011; Jenkins *et al.* 2003, 2008; Li *et al.* 2011; Spiller *et al.* 1998, 2003; Tamizifar *et al.* 2005). The main results of these studies can be found in Table 14.1. This hypocholesterolemic effect of almonds in both free-living and controlled study settings has been extensively reviewed by Berryman *et al.* (2011) and Kamil and Chen (2012). In summary, almonds lower low-density lipoprotein cholesterol (LDL-C) by 2.9% to 35.0% and total cholesterol (TC) by 1.5% to 35.0%, in a dose-dependent manner. However, the effect on high-density lipoprotein cholesterol (HDL-C) is still uncertain. Some studies have showed an increase in HDL-C by up to 8.1% (Foster *et al.* 2012) while others did not show any change (Abbey *et al.* 1994; Damasceno *et al.* 2011; Li *et al.* 2011; Spiller *et al.* 1998). The differences in subject ethnicity, study duration,

background diet, and almond dosage could all contribute to the inconsistency.

Table 14.1 Effect of almonds on cholesterol and lipoprotein profile in clinical interventions.

Design	Subjects	Duration (week)	Intervention	TG*	TC*	LDL-C*	HDL-C	Reference
Cross-over	Healthy, elevated LDL-C, 47.5 years, 26 F, 22 M	6	42.5 g/day almonds vs isocaloric muffin substitution (no almonds/day)	↓ 7.2%	↓ 5.1%	↓ 5.3%	↑ 1.7%	Berryman *et al.* 2015
Parallel	Overweight and obese, 37.5 years, 108 F	12	50 g/day almonds vs baseline	↓ 46.0%	↓ 35.0%	↓ 8.7%	–	Abazarfard *et al.* 2014
Parallel	Overweight and obese, 46.8 years, 112 F, 11 M	24 and 72	Hypocaloric or almond enriched diet vs baseline	↓ 12.6% ↓ 4.4%	↓ 4.4% ↓ 1.2%	↓ 4.7% ↓ 2.7%	↑ 0.1% ↑ 8.1%	Foster *et al.* 2012
Cross-over	Hypercholesterolemic, 50 years, 9 F, 9 M	4	Almonds (22% of total calories) vs baseline		↓ 6.4%	↓ 13.4%	–	Damasceno *et al.* 2011

Parallel	Healthy, 49.4 years, 38 F, 43 M	24	Almond (15% of total calories) vs habitual diet	↓ 1.0%	↓ 1.5%	↓ 2.9%	↑ 1.4%	Jaceldo-Siegl *et al.* 2011
Cross-over	T2DM, mild hyperlipidemia, 58 years, 11 F, 9 M	4	Almonds (20% of total calories) vs NCEP Step 2 diet	–	↓ 6.0%	↓ 11.6%	–	Li *et al.* 2011
Cross-over	Hyperlipidemic, 64 years, 12 F, 15 M	4	73 g whole almonds or 37 g whole almonds vs no almonds	↓ 12.2% ↓ 8.5%	↓ 3.6% ↓ 2.9%	↓ 2.8% ↓ 5.0%	↑ 1.5% ↑ 2.8%	Jenkins *et al.* 2008
Cross-over	Healthy, 41 years, 8 F, 8 M	4	Almonds (10% or 20% of calories) vs control diet	–	↓ 4.5%	↓ 7.1%	–	Jambazian *et al.* 2005
Cross-over	Hyperlipidemic, 56	4	25 g/day	↓ 7.2%	↓ 5.9%	↓ 12.3%	↑ 1.5%	Tamizifar *et al.*

	years, 13 F, 17 M		almonds vs NCEP Step 1 diet					2005
Cross-over	Healthy and mildly hypercholesterolemic, 41 years, 11 F, 14 M	4	68 g/day almonds vs NCEP Step 1 diet	–	↓ 4.4%	↓ 7.0%	–	Sabaté *et al.* 2003
Parallel	Hyperlipidemic, 60 years, 9 F, 16 M	4	Portfolio diet (with 16.6 g/1000 kcal almonds) vs NCEP Step 2 diet	–	↓ 26.6%	↓ 35.0%	–	Jenkins *et al.* 2003
Cross-over	Healthy, 43.5 years, 12 F, 10 M	6	66 g/day whole almonds or 35 g/day almond oil vs baseline	↓ 14.5% ↓ 15.3%	↓ 4.3% ↓ 4.5%	↓ 6.6% ↓ 7.0%	↑ 4.3% ↑ 6.9%	Hyson *et al.* 2002
Cross-over	Hypercholesterolemic, 64 years,	4	28 g/day or 56 g/	–	↓ 3.4% ↓	↓ 4.4% ↓	↑ 4.6% ↑	Jenkins *et al.* 2002

	12 F, 15 M		day almonds vs baseline		5.6%	9.9%	3.8%	
Parallel	Hypercholesterolemic, 53 years, 33 F, 12 M	4	100 g/day almonds vs control diet	–	↓ 15.6%	↓ 19.0%	–	Spiller *et al.* 1998
Parallel	Healthy, 41.3 years, 16 M	9	84 g/day raw almonds vs baseline	–	↓ 7.0%	↓ 10.3%	–	Abbey *et al.* 1994

*– no change; ↑ increase ↓ decrease.

F, female; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; M, male; NCEP, National Cholesterol Education Program; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triglycerides.

The fatty acid profile of almonds has been generally accepted as the primary mechanism responsible for the improvement in lipid profile (Berryman *et al.* 2011; Chen *et al.* 2006; Griel & Kris-Etherton 2007; Kamil & Chen 2012; Kris-Etherton *et al.* 2009; Sabaté *et al.* 2010). This notion is supported by a study by Hyson *et al.* (2002) illustrating that replacing half of their habitual fat (approximately 29% energy) for six weeks with either whole almonds or almond oil decreased LDL-C, TC, and triglycerides (TG) and increased HDL-C by a similar degree in 22 normolipemic men and women. Nishi *et al.* (2014) reported that incorporating almonds into a National Cholesterol Education Program (NCEP) Step 2 diet to replace ~10% or 20% of energy increased oleic acid and other unsaturated fatty acid contents in serum, total triglycerides, and nonesterified fatty acid fractions in hyperlipidemic adults. They further suggested that these changes in fatty acid profile could contribute to reduced risk for coronary

heart disease (CHD).

While the mechanism of action by which almonds improve the lipid profile has not been elucidated, it has been suggested that the positive effect of unsaturated fatty acids in almonds on hepatic very low-density lipoprotein (VLDL) production or/and VLDL lipolysis might contribute to a downstream reduction in LDL-C (Berryman *et al*. 2011; Foster *et al*. 2012; Spiller *et al*. 2003). Thus, the benefits of almonds on lipid profile can be ascribed mainly to their favorable lipid composition. Nevertheless, it should be noted that the magnitude of improvement is larger than the effect of almond lipids alone (Abbey *et al*. 1994; Berryman *et al*. 2015; Hyson *et al*. 2002; Jenkins 2002; Lovejoy *et al*. 2002; Sabaté *et al*. 2003; Spiller *et al*. 2003), suggesting that constituents other than lipids may make a contribution (Berryman *et al*. 2011; Griel & Kris-Etherton 2007; Sabaté *et al*. 2010). A more recent controlled feeding trial confirmed that almonds (42.5 g/day for six weeks) decreased LDL-C, TC, and TG and maintained HDL-C in patients with hypercholesterolemia, compared with a cholesterol-lowering control diet (Berryman *et al*. 2015),

In addition to the favorable lipid composition, other nutrients in almonds may play a role in the cholesterol lowering effect. As noted in the above nutrients section, almonds are a good protein source (Ahrens *et al*. 2005; Chen *et al*. 2006). Replacing dietary carbohydrates with proteins has been reported to be beneficial to LDL-C in both normolipidemic and hypercholesterolemic individuals (Appel *et al*. 2005), probably through an inhibition on hepatic VLDL secretion (Berryman *et al*. 2011). Almonds are a good source for dietary fiber. Of all tree nuts, almonds have the highest dietary fiber content. Its cholesterol lowering benefit has been well appreciated. The insoluble fibers in almonds help reduce LDL-C concentration by decreasing intestinal transit time and improving satiation (Hollis & Mattes 2007). Finally, phytosterols in almonds can help improve lipid profile by increasing cholesterol excretion and decreasing cholesterol absorption (Berryman *et al*. 2011).

14.3.2 Glucose Regulation

Almonds are regarded as a low glycemic index (GI) food because of their low available carbohydrate content, as well as their healthy lipid profile and high quantity of vegetable proteins, fibers, and magnesium. Therefore, almonds appear to be an appropriate food to be included in a diabetes management plan, and there have been some clinical trials examining the effect of almonds on glycemic control in healthy people and patients with diabetes (Table 14.2).

Table 14.2 Effect of almonds on glucose regulation and body weight control in clinical interventions.

Design	Subjects	Duration (week)	Intervention	Results*	Reference
Cross-over	Healthy, elevated LDL-C, 47.5 years, 26 F, 22 M	6	42.5–g/day almonds vs isocaloric muffin substitution (no almond)	• Abdominal fat: ↓ 3.4% • Leg fat: ↓ 1.3% • Body weight: -	Berryman *et al.* 2015
Parallel	T2DM, 50 years, 12 F, 9 M	12	42.5 g/day almonds vs no almond	• Carbohydrates consumption: ↓ 17.0% • Anthropometric profile:-	Sweazea *et al.* 2014
Parallel	Increased risk for T2DM, 39 years, 89 F, 48 M	4	43 g/day almonds with breakfast/lunch vs 43 g/day almonds	• Modulated postprandial glycemia: ↓ • Hunger and desire to eat: ↓ • Body weight: -	Tan & Mattes 2013

			alone as a morning or afternoon snack vs no almonds		
Cross-over	Impaired glucose tolerant, 39 years, 14 subjects	10	42.5 g whole almonds (WO), almond butter (AB), defatted almond flour (AF) or almond oil (AO) vs composite meal	• Daylong glucose, insulin and NEFA concentrations in WO and AO treatment groups: ↓	Mori *et al*. 2011
Cross-over	Diabetics, 3 F, 4 M, nondiabetics, 11 F, 2 M	12	28 g almonds vs baseline	• Postprandial glucose response: ↓ 30.3% in 4 participants with T2DM • Hemoglobin A1c: ↓ 4.2%	Cohen & Johnston 2011
Cross-over	T2DM, mild hyperlipidemia, 58 years, 11 F, 9 M	4	Almonds (20% of total calories) vs NCEP Step 2 diet	• Fasting insulin: ↓ 4.1% • Fasting glucose: ↓ 0.8% • HOMA: ↓ 9.2% • Body fat: ↓ 1.8% • Body weight and BMI: -	Li *et al*. 2011
Parallel	Overweight and obese, 53 years, 65	24	Low calorie diet + 84 g/day of	• Weight/BMI: ↓ 17.5% vs ↓ 10.9% • Waist circumference: ↓ 14.2% vs ↓ 10.0% • Fat mass: ↓ 30.3% vs ↓ 19.6%	Wien *et al*. 2003

	adults		almonds vs complex carbohydrates		
Cross-over	T2DM 35 years, 17 F, 13 M	4-	High-fat, high-almond (37% total fat, 10% from almonds) vs low-fat, high-almond (25% total fat, 10% from almonds) vs high-fat control vs low-fat control	• Glycemic status: - • Insulin sensitivity:- • HbA1c -	Lovejoy *et al.* 2002

* – no change; ↑ increase ↓ decrease.

BMI, Body Mass Index; F, female; HOMA, Homeostasis Model Assessment; LDL-C, low-density lipoprotein cholesterol; M, male; NCEP, National Cholesterol Education program; NEFA, nonesterified fatty acids; T2DM, type 2 diabetes mellitus.

Almonds are capable of modulating the GI of co-consumed foods. Josse *et al.* (2007) found that almonds decreased the GI of white bread in a dose-dependent manner. The GI-modulating effect can be extended to meals containing a more complex nutrient profile than carbohydrate-rich white bread. In a four-week randomized, parallel designed trial with 137 healthy adults consuming almonds (43 g/day) with breakfast or lunch or alone as morning or afternoon snacks, Tan and Mattes (2013) observed a decrease in the postprandial glucose response 60 minutes after ingestion.

Glycemic control is crucial to those who have impaired glucose regulation, e.g. patients with diabetes and metabolic syndrome. Cohen and Johnston (2011) reported in an acute trial that almonds (1 serving, 28 g) consumed immediately before a starchy meal significantly reduced postprandial glycemic response in patients with type 2 diabetes mellitus. They also found in a longer term study that after 12 weeks of almond consumption (28 g/day for five days/week), HbA1c was significantly reduced by 4% compared to the baseline. Li *et al*. (2011) also noted in a controlled feeding study that replacing 20% of dietary calories with almonds led to significant decreases in fasting blood glucose, insulin, and homeostatic model assessment (HOMA) in patients with type 2 diabetes. Interestingly, Lovejoy *et al*. (2002) did not find any change in glycemic status, insulin sensitivity, and HbA1c in patients with type 2 diabetes who consumed almonds to replace 10% daily energy need for four weeks. The conflicting results might be attributed to almond dose, diabetes duration, and study design.

The constituents in almonds contributing to the blood glucose-modulating effect have not been fully elucidated. Mori *et al*. (2011) suggested that the modulation is most likely due to the high unsaturated fat content. This suggestion was based on the results of a human study showing that almond oil, rather than almond butter and defatted almond flour, exhibited the same degree of suppressive effect on postprandial glucose response as whole almond. The low and delayed postprandial blood glucose response might be a consequence of almond lipid-mediated reduction in the breakdown rate of complex carbohydrates through its inhibitory effect on gastric emptying (Tan & Mattes 2013). In addition to the almond lipids, polyphenols and phytates in whole almonds can inhibit carbohydrate digestive enzymes, an action resulting in a decrease in overall glucose absorption and subsequent blood glucose surge (Lo Piparo *et al*. 2008; Yoon *et al*. 1983). Thus, almonds may help decrease the incidence of metabolic syndrome, type 2 diabetes, and cardiovascular disease via the bioactions of glucoregulation because lowering postprandial glucose excursion could decreases the risk of oxidative damage to lipids and proteins (Jenkins *et al*. 2006).

14.3.3 Antiinflammation

Inflammation is one of the mechanisms involved in the development and progression of atherosclerosis and insulin resistance (Danesh *et al*. 2004; Festa *et al*. 2002). Inflammatory markers, such as C-reactive protein (CRP), interleukin-6 (IL-6), fibrinogen, vascular cell adhesion molecule-1 (VCAM-1), and intracellular adhesion molecule-1 (ICAM-1), have been identified as independent predictors for cardiovascular disease or type 2 diabetes (Asegaonkar *et al*. 2011; Luc *et al*. 2003; Pradhan & Ridker 2002; Pradhan *et al*. 2001; Soinio *et al*. 2006; Zhang *et al*. 2009). Due to their favorable nutrient profile, almonds have been shown to diminish inflammation via direct and indirect mechanisms (e.g. ameliorating glucose dysregulation).

Sweazea *et al*. (2014) determined the effect of almonds on the biomarkers of diabetes and cardiovascular disease in patients with type 2 diabetes in a randomized, parallel design study. They found that after consumption of 42.5 g almonds/day, 5 days/week for 12 weeks, CRP was reduced by ~30% ($p = 0.029$) compared to no dietary change, and IL-6 and tumor necrosis factor (TNF)-α were not affected. In agreement with this study, Liu *et al*. (2013) illustrated in a randomized, cross-over, controlled feeding trial with Chinese patients with type 2 diabetes and mild hyperlipidemia that in comparison with the NCEP Step 2 diet, the incorporation of almonds to replace 20% daily calories significantly decreased IL-6 and CRP and tended to decrease TNF-α (Liu *et al*. 2013). In addition, Rajaram *et al*. (2010) reported in a randomized, controlled, cross-over feeding study with 25 healthy Americans that compared to a nut-free diet, almonds replacing 10% and 20% of daily calories lowered CRP and E-selectin in a dose-independent manner. In contrast, Estruch *et al*. (2006) found in a PREDIMED study of 772 free-living asymptomatic adults that three months consumption of a Mediterranean diet including mixed nuts (30 g/day of walnuts, hazelnuts, and almonds) did not change CRP, but reduced circulating IL-6, ICAM-1, and VCAM-1. More information about the results of these studies is given in Table 14.3. The apparent inconsistency in inflammatory responses to almond or nut consumption indicates the complexity of the

inflammatory network and suggests inclusion of multiple inflammatory biomarkers to test hypotheses in clinical trials.

Table 14.3 Effect of almonds on inflammation and antioxidation in clinical interventions.

Design	Subjects	Duration (week)	Intervention	Results*	Reference
Parallel	T2DM, 50 years, 9 M, 12 F	12	42.5 g/day almonds vs no almonds	• CRP: ↓ 27.8% • IL-6: - • TNF-α: -	Sweazea *et al.* 2014
Cross-over	T2DM, 58 years, 9 M, 11 F	4	56 g/day almonds (20% of calories) vs control diet	• CRP: ↓ 10.3% • IL-6: ↓ 10.3% • TNF-α: ↓ 15.7%	Liu *et al.* 2013
Cross-over	Healthy, 37.5 years, 14 F, 11 M	4	Almonds (10% or 20% of calories) vs baseline	• CRP: ↓ 9.1%, ↓ 4.5% • IL-6: - • E-selectin: ↓ 1.5%, ↓ 7.7%	Rajaram *et al.* 2010
Parallel	High cardiovascular risk, 67.5 years, 433 F, 339 M	12	Mediterranean diet, mixed nuts (30 g/day walnuts, hazelnuts, and almonds) vs baseline	• CRP: - • IL-6: ↓ 1.3 ng/mL • ICAM-1: ↓ 97 ng/mL • VCAM-1: ↓ 167 ng/mL	Estruch *et al.* 2006
Parallel	20 M (56 years), 20	4	50 g/day almonds	• Plasma α-tocopherol/cholesterol ratio: ↑ 3.0% • Flow-mediated dilation: ↑	Choudhury *et al.*

	M (23 years), 20 M (27 years) with two or more CV risk factors, 15 M above 18 years (control)		vs habitual diet	• Systolic blood pressure: ↓ • Vascular function: ↑	2014
Cross-over	T2DM, mild hyperlipidemia, 58 years, 11 F, 9 M	4	Almonds (20% calories) vs NCEP Step 2 diet	• Plasma α-tocopherol: ↑ 26.8%	Li *et al.* 2011
Cross-over	Healthy soldiers, smokers, 21.5 years, 60 M	4	84 g/day almonds vs 120 g/day pork	• Serum α-tocopherol: ↑ 9.7% • Superoxide dismutase: ↑ 35.1% • Glutathione peroxidase: ↑ 16.4% • Urinary 8-OHdG: ↓ 27.4% • Urinary malondialdehyde: ↓ 21.1% • DNA strand breaks: ↓ 34.4%	Li *et al.* 2007
Cross-over	Healthy, 35.5 years, 8 F, 7 M	5 study sessions, 4 h/session	2 bread control meals 3 test meals: almonds and bread; parboiled rice;	• Serum protein thiol: ↑ • Oxidative protein damage: ↓	Jenkins *et al.* 2006

		instant mashed potatoes		
Cross-over	Healthy, 41 years, 8 F, 8 M	Almonds (10% or 20% of calories) vs control diet	• Blood α-tocopherol: ↓ 19.4%; ↓ 28.5%	Jambazian *et al.* 2005

* - no change; ↑ increase ↓ decrease.

CRP, C-reactive protein; CV, cardiovascular; F, female; ICAM, intracellular adhesion molecule; IL, interleukin; M, male; NCEP, National Cholesterol Education Program; T2DM, type 2 diabetes mellitus; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule.

It is quite challenging to characterize in a whole food concept which nutrients are responsible for decreasing inflammation. Antioxidant vitamins, fiber, L-arginine, magnesium, and phytochemicals in almonds may all work together to exert antiinflammatory actions (Calder *et al*. 2009; Casas-Agustench et al 2010; Jiang *et al*. 2006; Lucotti *et al*. 2009; Salas-Salvadó *et al*. 2008; Singh *et al*. 2005; Wells *et al*. 2005). Furthermore, the observed antiinflammatory effect may simply be secondary to the improvements in blood cholesterol and glucose in patients with metabolic disorders. Thus, more studies are warranted to elucidate the mechanism of action for the reductions in inflammatory biomarkers.

14.3.4 Antioxidation

Almonds contain a variety of antioxidant phytochemicals, including phenolic compounds and α-tocopherol, which have been inversely linked to risk factors for chronic diseases, such as cardiovascular diseases and diabetes (Kendall *et al*. 2010; Ros 2009). However, it should be noted that, as for the above-mentioned antiinflammatory benefits, the antioxidative effects of almonds may be due to the reduction in oxidative stress secondary to the overall improvement in wellbeing. The benefits of almonds

in oxidative stress status have been demonstrated in healthy individuals and patients with chronic disease, and such evidence has been reviewed by Chen *et al*. (2006), Mirrahimi *et al*. (2011), and Kamil and Chen (2012). As almonds are one of the richest sources of vitamin E, their consumption has been linked to elevated α-tocopherol status in a dose-dependent manner (Jambazian *et al*. 2005).

As LDL oxidation plays a significant role in atherogenesis, one approach to enhancing the resistance of LDL to oxidation is to augment lipophilic antioxidants in LDL particles. As anticipated because of the high α-tocopherol content, almond consumption is linked to elevated resistance of LDL to oxidation in hyperlipidemic and normolipidemic people (Kamil & Chen 2012). Polyphenols in almonds, mostly flavonoids, such as flavanols and flavonol glycosides in the skin, may also contribute to increasing the antioxidant defense network by acting as antioxidants or by modulating endogenous antioxidant defenses. The predominant flavonoid present in almonds is isorhamnetin rutinoside (Milbury *et al*. 2006). It is worth noting that absorbed polyphenols might work with other antioxidants such as vitamin C and E in a synergistic manner to protect susceptible molecules against radical attack (Chen *et al*. 2005).

Besides protection of DNA and lipid, almond nutrients can protect proteins against radical attack or conjugation with aldehydes. For example, Jenkins *et al*. (2006) observed that protein thiol concentration in serum was increased following almond ingestion, suggesting less oxidative protein damage. As almond consumption is associated with reduced glycemic excursion in healthy people and improved glucoregulation in patients with type 2 diabetes, amelioration of oxidative stress may be secondary to the improvement in hyperglycemia (Josse *et al*. 2007; Li *et al*. 2011).

Normal endothelial functions are important to prevent/protect against development and progression of atherosclerosis. Abnormalities in endothelial function originate from many factors, with oxidative stress and inflammation being the best established. Choudhury *et al*. (2014) reported for the first time that almonds (50 g/day for four weeks) improved endothelial function, which was assessed using flow-mediated dilation technique in

asymptomatic healthy young and middle-aged men with two or more cardiovascular risk factors. They also noted that systolic blood pressure was improved by almonds. While the exact mechanism of action for the improvement remains to be elucidated, the effect of almond nutrients on oxidative stress and inflammation may have some contributions in this regard (see Table 14.3).

The overall data suggest that α-tocopherol and polyphenols as the main antioxidants work together in an additive manner to protect lipid, DNA, and protein from oxidation. It should be noted that almonds may also help decrease oxidative stress status via improvements in hyperlipidemia and hyperglycemia which are associated with the production of reactive oxygen species.

14.3.5 Body Weight Control

Almonds have historically been perceived as a food causing unwanted weight gain because of their high fat content. However, this perception is changing because their consumption does not link with weight gain but rather is associated with reduced Body Mass Index (BMI) and their inclusion in weight-maintaining diets is therefore recommended (Bes-Rastrollo *et al*. 2009).

According to the reviews of Sabaté (2003), Rajaram and Sabaté (2006), and Kamil and Chen (2012), the inclusion of almonds in the diet without any advice or restriction resulted in no significant change in body weight or BMI. These results suggest that the additional energy derived from almonds was displaced by reduced consumption of other foods, a food displacement effect. This mechanism is further substantiated by a 12-week randomized, parallel-arm controlled clinical trial, in which patients with type 2 diabetes in the almond group tended to consume fewer carbohydrates (Sweazea *et al*. 2014). The displacement effect can be ascribed to increased satiety and fullness and decreased hunger after almond consumption, which are good attributes for weight loss and maintenance. Cassady *et al*. (2009) observed that as a hard food, almonds could extend mastication time, which in turn elevates satiety, suppresses hunger, and modulates the release of gut hormones such as cholecystokinin, glucagon-like peptide-1,

and peptide YY. Similarly, Hull *et al*. (2015) found that adding almonds (28 or 42 g/day) as a mid-morning snack for three days decreased the amount of foods consumed during lunch and dinner, whose calories were equivalent to the 173 and 259 kcals consumed as almonds. Further, the subjective appetite ratings measured between the snack and lunch were higher in a dose-dependent manner. Although half the weight of almonds is lipids, these lipids are not so bioaccessible and remain unavailable during the whole digestion process because of the structural barriers of cell walls that impede the penetration of digestive enzymes (Berry *et al*. 2008; Grundy *et al*. 2015).

Although it was developed more than 100 years ago by Atwater and Bryant (1900), the Atwater factor system is still widely employed to estimate the energy value of foods. However, Novotny *et al*. (2012) has proved with very astonishing evidence that the Atwater factor, when applied to almonds, resulted in a 32% overestimation of their measured energy content, which resulted from unabsorbed lipids. All of these results suggest that almonds can delay nutrient absorption and maintain satiety/suppress hunger and have a low metabolized energy content.

The benefits of almonds on body weight can extend to body composition. During weight loss, the reduction in body fat, especially in the central abdominal area, is the most beneficial to health compared to the loss of subcutaneous fat. In a 24-week trial with 65 overweight and obese adults, a low-calorie diet enriched with 84 g/day of almonds reduced body weight/BMI, waist circumference, and fat mass significantly more than the low calorie control diet (Wien *et al*. 2003)., Reduction in body fat was consistently noted in Chinese patients with type 2 diabetes (Li *et al*. 2011). In a more recent randomized, cross-over, controlled feeding study on 48 people with high LDL-C, a cholesterol-lowering diet with and without addition of almonds (42.5 g/day) decreased abdominal fat and leg fat, despite no difference in body weight between the two dietary groups (Berryman *et al*. 2015). While the mechanism(s) by which almond constituents reduced abdominal fat remains to be explored, MUFA in almonds may enable the redistribution of central body fat (Paniagua *et al*. 2007). More detailed information is presented in Table 14.2.

According to a World Health Organization report (WHO 2014), more than 1.4 billion adults (≥ 20 years) were overweight in 2008 and more than 40 million children (under age five) were overweight or obese in 2012. The epidemic of overweight and obesity is a global problem because they are closely associated with increasing prevalence of the metabolic syndrome, type 2 diabetes, and cardiovascular diseases, which have serious implications for healthcare systems and the financial burden for individuals and countries (Mozumdar & Liguori 2011). Health and nutrition educational programs based on the research data are vital to control obesity and its related diseases. With the growing body of evidence on health benefits and body weight loss/maintenance, almonds can be included in healthy diets to control and maintain body weight because of their low metabolized energy, hunger suppression, and appealing taste.

14.3.6 Prebiotics

Dietary fiber intake is continually encouraged in all populations because fiber intake remains low, insufficient to reach the level at which its maximum health benefits are achieved. Gut health has drawn a lot of public attention recently, especially with emerging evidence showing the link between gut health and diseases in organs distant from the gut. One of the main research areas is to establish the impact on the gut microbiome of prebiotics and probiotics which foster the growth of beneficial microbes and suppress harmful ones. A prebiotic is a nutrient, compound or food which is resistant to human digestive enzymes, can benefit the growth of beneficial bacteria, and promotes host wellness and health (Gibson *et al.* 2004; Pineiro *et al.* 2008). Dietary fibers and resistant starches (typically polysaccharides, such as pectins and xylans) are well recognized as prebiotics, and they are degraded by bacterial enzymes but resistant to pancreatic enyzmes (Gibson *et al.* 2010). Almonds are one of the most fiber-rich foods. With 3.4 g fiber per 28 g serving, almonds provide a significant amount of fiber for microbial fermentation in the gut. The total fiber content of whole almonds is among the highest (12%) of all the edible nuts (Mandalari *et al.* 2008). The total dietary fiber of almond skin (by-product of the almond-processing industry) is approximately 45%

wet weight (w/w), most of this being insoluble fiber with 3–4% soluble fiber (Mandalari *et al*. 2010a,b). Using an *in vitro* fermentation bioreactor, a high amount of almond skin cellulose was found at different stages of fermentation in the large bowel and the bifidobacteria and *Eubacterium rectale* populations were increased, suggesting that almond skins might have prebiotic properties (Mandalari *et al*. 2008).

Liu *et al*. (2014) demonstrated the prebiotic effect of almonds in a human study. They found that the six-week consumption of roasted almonds (56 g/day) or almond skins (10 g/day) increased the populations of *Bifidobacterium* spp. and *Lactobacillus* spp. in 48 healthy adult volunteers and decreased the pathogen *Clostridium perfringens*. Further, almonds or almond skins increased activity of β-galactosidase, which is mainly synthesized by bifidobacteria and lactobacilli, and decreased activities of β-glucuronidase, nitroreductase, and azoreductase, which are synthesized by harmful bacteria, suggesting a favorable change in the microbial profile. In contrast, Ukhanova *et al*. (2014) did not find a marked impact of almond consumption (up to 85 g/day for 18 days) on microbiota, particularly in lactic acid bacteria, in a randomized, controlled, cross-over feeding study with healthy adults, even though there was a decrease in *Firmicutes* bacterium DJF VP 44 and *Clostridium* sp. ASF 396. Thus, the prebiotic effect of almonds or their constituents may depend on the duration of consumption. The complexity of the gut's microbial ecosystem and a lack of definitive means for its characterization are also underlying factors for the inconsistency (Di Bella *et al*. 2013).

In addition to dietary fibers, polyphenols present in almonds skins could have a prebiotic effect via their microbial modulating action. However, their impact on the gut microbiota remains to be elucidated. Nevertheless, gut bacteria are capable of metabolizing polyphenols, especially transforming larger polyphenols to simple phenolic acids. Such small phenolic acids derived from bacterially mediated metabolism of polyphenols in almond skins have been detected in plasma and urine (Urpi-Sarda *et al*. 2009). This interplay between almond polyphenols and gut microbiota can have significant implications for health in the gut and the whole body because this relationship can be associated with reduction in

harmful microbes, production of antimicrobial substances, and modulation of metabolic and autoimmune diseases (Round & Mazmanian 2009). Thus, the use of almonds in the development of prebiotic foods appears to be an appealing strategy for health prevention and promotion.

14.4 Development of Functional Foods with Almonds

Recently, there has been growing interest in the development of functional ingredients and foods, nutraceuticals, and dietary supplements (Shahidi 2009). The term "functional food" was first mentioned in Japan in 1984 in the context of a food related to nutrition, modulation of physiological systems, sensory satisfaction, and fortification. More recently, functional foods have been defined as food products with special constituents that promote biological, molecular, and physiological effects benefiting health (Bigliardi & Galati 2013; Hardy 2000). With the astronomical increase in healthcare costs and aging populations, functional foods may have a place in health maintenance and promotion (Shahidi 2009).

The actions of a functional food rely on the bioactives naturally present in the product, are artificially formulated using appropriate technologies, or both. Development of functional foods began initially with the fortification of essential nutrients, such as vitamin C and E, folic acid, zinc, iron, calcium, and so on. In the last decade, the focus has evolved to adding novel constituents, such as omega-3 fatty acids, phytosterol, and soluble fiber (β-glucan), and developing foods with multiple health benefits. Further, a wide range of food products, such as breakfast cereals, snacks, beverages, and supplements, have been employed as platforms to deliver functional ingredients or nutrients (Sloan 2000, 2002, 2004, 2014).

Development of functional foods is a complex, challenging process with a critical need to attain product acceptance by consumers and necessary approvals from regulatory authorities (Day *et al*. 2009; Jones & Jew 2007). Consumer acceptance is one

of the top priorities in the development of functional foods because characteristics of quality attributes, like texture and flavor, can be affected even though the potential negative effects can be minimized with the use of other quality enhancement ingredients and techniques (Day *et al*. 2009). In particular, sensory attributes such as taste, color, aroma, and texture are important elements with a great influence on consumer acceptance. Stability of functional ingredients/constituents in finished products is also critical to success. Technologies that stabilize bioactive compounds are in development, such as microencapsulation (envelopment of small solid particles, liquid droplets or gases in a coating), edible films (carry active ingredients that can reduce the risk of pathogen growth on the food surface or specific nutrients beneficial to humans), and vacuum impregnation (introduces desirable solutes into the porous structures of foods) (Betoret *et al*. 2011; Bigliardi & Galati 2013).

In July 2003, the FDA approved a health claim stating, “Scientific evidence suggests but does not prove that eating 1.5 ounces per day of most nuts, such as almonds, as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease” (FDA 2014). Food products with health claims attesting to functional capacity and increased quality of life in the general population are well accepted by consumers (Jones & Jew 2007). Almonds and their processing by-products can provide a wide range of ingredients for the development of functional foods because of their functional constituents (monounsaturated fat, magnesium, α-tocopherol, fiber, polyphenols, riboflavin, and other micronutrients).

According to a review by Siró *et al*. (2008), the most notable platforms for functional products include prebiotics and probiotics, beverages, cereals, bakery products, spreads, meats, and eggs. We believe that almonds and their derivatives, such as almond paste, milk, and oil, could be added in most of these categories as ingredients to enhance functionality and bioactive content of functional foods. Bakery products present an ideal vehicle by which functionalities of bioactives or nutrients can be delivered to the consumer in an acceptable food (Siró *et al*. 2008). Almonds in different forms have been traditionally used in many bakery and confectionery products such as cakes, pies, cookies, and breads.

The introduction of almonds into bakery products has grown by 13% in Europe and North America (California Almonds 2014), an increase most likely attributed to the health benefits substantiated by a growing body of clinical evidence. In addition to bakery and confectionery products, almonds have been formulated into breakfast cereals, snack bars, almond chocolate, and nut mixtures as a snack. Further, almond butter, which contains significantly more fiber, calcium, and potassium than sunflower seed or peanut butter, has become a new alternative for those who are allergic to peanuts (Thomas & Gebhardt 2010). It should be noted that almond butter should contain a minimum of 90% almonds and be prepared by grinding shelled, blanched or unblanched, raw or roasted almonds, to which salt, honey, evaporated cane syrup, corn maltodextrin, flax seed, wheatgerm, cocoa powder, cocoa butter or vanilla may be added as ingredients (USDA 2011). Palm or peanut oil can be used as a stabilizing agent.

Almond skins are rich in polyphenols and dietary fiber and could be considered a functional food ingredient, as well as a natural antioxidant preservative added to control oxidative processes in more oxidation-prone foods (Garrido *et al.* 2008). During almond processing, skins are produced as a by-product. While they are a valuable ingredient because of their polyphenol and fiber content, their true value has not been fully realized in the arena of foods, nutraceuticals, and pharmaceuticals. Dietary fibers have many roles in health promotion and prevention, e.g. increasing cholesterol elimination, maintaining glucose regulation, and modulating gut microbiota. Thus, the incorporation of almond skins into bakery and cereal products can enhance their functional characteristics. They can also be added to granola mix, rice, mashed potatoes, pastas, salads and salad dressings, cereal bars, crackers, yogurts, fermented beverages, juices, and bakery products such as muffins, cookies, pancakes, and waffles. Finally, they can be lyophilized, powdered, and then sold as a functional ingredient to food manufacturers or as a functional food to consumers. These functional foods made with almond skins could provide an array of health benefits, for example lowering blood cholesterol, maintaining/decreasing blood glucose, enhancing antioxidant defense and immunity, and modulating gut microbiota. Furthermore, the polyphenols present in the skins could be used as

a potential natural antimicrobial agent in the food preservative market (Mandalari 2012). The photoprotective potential of polyphenols and other constituents in almond skins have also been reported, suggesting that almond nutrients can be used to develop products for skin health (Evans *et al*. 2013).

Almond oil displays many health benefits due to its rich content of α-tocopherol and oleic acid in antioxidation and cholesterol reduction for CVD prevention. In addition, almond oil can serve as an ingredient in skin (as an emollient) and hair care products. It could be used in the production of salad dressings, mayonnaise, whipped cream, and cake fillings and toppings to improve their functional characteristics. However, one downside is that production costs will be augmented because of the relatively higher cost of almond oil compared to other vegetable oils on the market.

Almond flour is made with blanched almonds, whereas almond meal can be made with either whole or blanched almonds. Almond flour or meal can be used to replace wheat flour in products such as cakes, waffles, cookies, pancakes, and breads. This replacement benefits the products by reducing carbohydrate content whose consumption is generally linked to the development or progression of metabolic disorders and by gaining the health benefits of almonds. Further, patially replacing wheat flour with almond meal can add texture and flavor to the products. Almond meal can also be used in place of bread crumbs in meatballs or as a coating for fish and chicken. Almond meal is a gluten-free product and thus is an interesting alternative for people with gluten-related disorders.

Almond milk has become popular and is an alternative to dairy milk especially for consumers with lactose intolerance and dairy protein allergy. With its favorable taste and nutrition values, the demand for almond milk has been increasing. Similar to almond milk, the demand for soymilk is escalating. Almond milk could serve as the base ingredient for production of new nondairy fermented products with probiotic bacteria and functional features. Bernat *et al*. (2015) evaluated the fermentative process of almond milk using a mixed culture of *L. reuteri* and *S. thermophilus* and found that the fermentation induced an increase in the viscosity, luminosity, and whiteness values of the almond milk. High

probiotic survivals were also observed in the fermented almond milk after submitting the product to *in vitro* digestion, enhancing the product value as a probiotic.

Processed meat products can be a valuable vehicle in delivery of functional ingredients (Olmedilla-Alonso *et al*. 2006). Such an approach can decrease the unhealthy attributes of meat products, e.g. saturated fats and sodium. While there is currently no application using almonds or their derivatives, walnuts were incorporated into restructured beef steak to enhance sensory and healthy attributes (Jiménez Colmenero *et al*. 2003). Thus, the incorporation of nuts in meat products can be a means to confer their potential heart-healthy benefits to generally unhealthy but popular products. Further research is warranted to enhance understanding of the interactions between constituents in added nuts and meat products with the concerns of food safety and texture change being taken into account (Fernández-Ginés *et al*. 2005).

Functional foods are one of the growing segments of the food industry with the potential to improve health and help to slow the increase in healthcare costs. New approaches to formulating functional ingredients or bioactives in functional foods are being undertaken by the academic and private sectors. Most importantly, the route to success in development of a functional food must start from selection of functional ingredients or bioactives and appropriate vehicles and then determine consumer acceptability and stability of functional nutrients (Betoret *et al*. 2011). Further, studies must be undertaken to elucidate any changes in absorption, disposition, metabolism, and excretion of functional nutrients in the new food matrixes, as well as bioefficacy. Finally, in the era of personal medicine, nutrigenomics must be taken into consideration when a functional food is developed for general or specific populations (Hasler 2002).

14.5 Conclusion

Among many recent innovations in the food industry, functional foods are recognized as one of the most interesting areas with

great growth potential. Development of new functional foods appears to follow a market trend, which begins with an influx of new research data showing health benefits of foods or nutrients beyond their standard nutrition value. Further, the growing interest of consumers in functional foods for the promotion of overall wellbeing and reduction in risk of chronic diseases drives the development of these foods and their commercialization. Almonds are one type of nut whose consumption was associated with a reduced risk for mortality in the Physicians' Health Study (Hshieh *et al.* 2015). While almonds are rich in calories, they have become recognized as a food with multiple health attributes due to their nutrition profile which is rich in oleic acid, fiber, α-tocopherol, magnesium, riboflavin, and polyphenols. The growing body of clinical evidence has shown that their consumption is linked with reduction in blood cholesterol, improvement in glucose regulation and antiinflammation, and amelioration of oxidative stress in those who are healthy or have chronic diseases.

With their health benefits and taste, texture, and flavor characteristics, almonds are a great ingredient to be formulated in functional foods. They can be incorporated into functional foods in diverse forms, for example as whole almonds (slices, flour, and paste), skins, milk, and oil. Even though almonds are traditionally used in bakery and confectionery products or consumed as a snack, almonds or their derivatives can be formulated into functional foods, e.g. cereal products, processed meats, and can replace unfavorable ingredients, such as refined wheat flour or oil.

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